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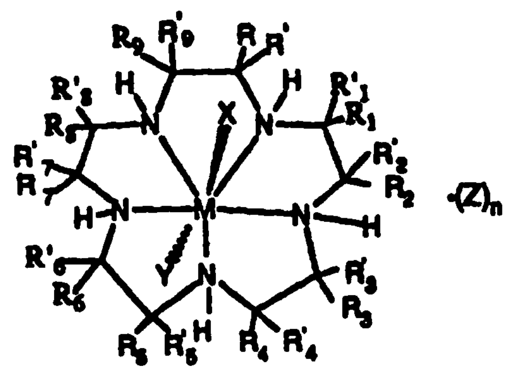
(57) Abstract

Bioconjugates of low molecular weight mimics of superoxide dismutase (SOD) represented by formula (I) wherein R, R', R_1, R'_1, R_2, R'_2, R_3, R'_3, R_4, R'_4, R_5, R'_5, R_6, R'_6, R_7, R'_7, R_8, R'_8, R_9, R'_9, X, Y, Z, M and n are as defined herein, useful as therapeutic agents for inflammatory disease states and disorders, such as ischemic/reperfusion injury, stroke, atherosclerosis, and all other conditions of oxidant-induced tissue damage or injury.



(I)

BIOCONJUGATES OF MANGANESE OR IRON COMPLEXES OF NITROGEN-CONTAINING MACROCYCLIC LIGANDS EFFECTIVE AS CATALYSTS FOR DISMUTATING SUPEROXIDE

BACKGROUND OF THE INVENTION

This present invention relates to compounds effective as catalysts for dismutating superoxide. This invention relates to manganese or iron complexes of nitrogen-containing fifteen-membered macrocyclic ligands which catalytically dismutate superoxide. In another aspect, this invention relates to manganese or iron complexes of nitrogen-containing fifteen-membered macrocyclic ligands which are conjugated to a targeting biomolecule.

2. Related Art

The enzyme superoxide dismutase catalyzes the conversion of superoxide into oxygen and hydrogen peroxide according to equation (1) (hereinafter referred to as dismutation). Reactive oxygen metabolites derived from superoxide are postulated to contribute to the tissue pathology in a number of

$$\dot{O_2}^- + \dot{O_2}^- + 2H^+ \rightarrow O_2 + H_2O_2 \qquad (1)$$

inflammatory diseases and disorders, such as reperfusion injury to the ischemic myocardium, inflammatory bowel disease, rheumatoid arthritis, osteoarthritis, atherosclerosis, hypertension, metastasis, psoriasis, organ transplant rejections, radiation-induced injury, asthma, influenza, stroke, burns and trauma. See, for example, Bulkley, G.B., Reactive oxygen metabolites and reperfusion injury: aberrant triggering of reticuloendothelial function, *The Lancet*, Vol. 344, pp. 934-36, October 1, 1994; Grisham, M.B., Oxidants and fr e radicals in inflammatory bowel disease, *The Lancet*, Vol. 344, pp. 859-861, September 24, 1994; Cross, C.E. et al., Reactive oxygen species and the lung, *The*

Lancet, Vol. 344, pp. 930-33, October 1, 1994; Jenner, P., Oxidative damage in neurodegenerative disease, *The Lancet*, Vol. 344, pp. 796-798, September 17, 1994; Cerutti, P.A., Oxy-radicals and cancer, *The Lancet*, Vol. 344, pp. 862-863, September 24, 1994 Simic, M. G., et al, Oxygen Radicals in Biology and Medicine, Basic Life Sciences, Vol. 49, Plenum Press, New York and London, 1988; Weiss J. Cell. Biochem., 1991 Suppl. 15C, 216 Abstract C110 (1991); Petkau, A., Cancer Treat. Rev. 13, 17 (1986); McCord, J. Free Radicals Biol. Med., 2, 307 (1986); and Bannister, J.V. et al, Crit. Rev. Biochem., 22, 111 (1987). The above-identified references from *The Lancet* teach the nexus between free radicals derived from superoxide and a variety of diseases. In particular, the Bulkley and Grisham references specifically teach that there is a nexus between the dismutation of superoxide and the final disease treatment.

It is also known that superoxide is involved in the breakdown of endothelium-derived vascular relaxing factor (EDRF), which has been identified as nitric oxide (NO), and that EDRF is protected from breakdown by superoxide dismutase. This suggests a central role for activated oxygen species derived from superoxide in the pathogenesis of vasospasm, thrombosis and atherosclerosis. See, for example, Gryglewski, R.J. et al., "Superoxide Anion is Involved in the Breakdown of Endothelium-derived Vascular Relaxing Factor", *Nature*, Vol. 320, pp. 454-56 (1986) and Palmer, R.M.J. et al., "Nitric Oxide Release Accounts for the Biological Activity of Endothelium Derived Relaxing Factor", *Nature*, Vol. 327, pp. 523-26 (1987).

Clinical trials and animal studies with natural, recombinant and modified superoxide dismutase enzymes have been completed or are ongoing to demonstrate the therapeutic efficacy of reducing superoxide levels in

the disease states noted above. However, numerous problems have arisen with the use of the enzymes as potential therapeutic agents, including lack of oral activity, short half-lives *in vivo*, immunogenicity with nonhuman derived enzymes, and poor tissue distribution.

The manganese or iron complexes of nitrogen-containing fifteen-membered macrocyclic ligands that are low molecular weight mimics of superoxide dismutase (SOD) are useful as therapeutic agents and avoid many of the problems associated with SOD enzymes. However, it would be desirable to be able to direct the SOD mimics to a desired target in the body where the compound can be concentrated for optimal effect. Without some way to render the compounds "targeting", increased dosages are sometimes necessary in order to obtain an efficacious concentration at the site of interest. Such increased dosages can sometimes result in undesirable side effects in the patient.

It has now been found that the macrocycles or manganese or iron complexes of the present invention can be attached, i.e. conjugated, to one or more targeting biomolecule(s) via a linker group to form a targeting biomolecule-macrocycle or targeting biomolecule-manganese complex conjugate.

SUMMARY OF THE INVENTION

It is an object of the invention to provide bioconjugates of manganese or iron complexes of nitrogen-containing fifteen-membered macrocyclic ligands that are low molecular weight mimics of superoxide dismutase (SOD) which are useful as therapeutic agents for inflammatory disease states or disorders which are mediated, at least in part, by superoxide. It is a further object of the invention to provide bioconjugates of manganese (II) or iron (III) complexes of nitrogen-

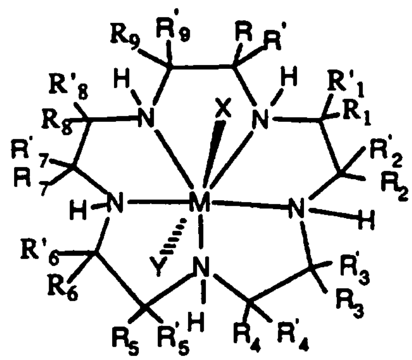
containing fifteen-membered macrocyclic ligands which are useful as magnetic resonance imaging (MRI) contrast agents having improved kinetic stability, improved oxidative stability and improved hydrogen bonding. It is yet a further object of the invention to provide bioconjugates of manganese or iron complexes of nitrogen-containing fifteen-membered macrocyclic ligands that can be targeted to a specific site in the body.

According to the invention, bioconjugates of manganese or iron complexes of nitrogen-containing fifteen-membered macrocyclic ligands are provided wherein (1) one to five of the "R" groups are attached to biomolecules via a linker group, (2) one of X, Y and Z is attached to a biomolecule via a linker group, or (3) one to five of the "R" groups and one of X, Y and Z are attached to biomolecules via a linker group; and biomolecules are independently selected from the group consisting of steroids, carbohydrates, fatty acids, amino acids, peptides, proteins, antibodies, vitamins, lipids, phospholipids, phosphates, phosphonates, nucleic acids, enzyme substrates, enzyme inhibitors and enzyme receptor substrates and the linker group is derived from a substituent attached to the "R" group or X, Y and Z which is reactive with the biomolecule and is selected from the group consisting of $-NH_2$, $-NHR_{10}$, -SH, -OH, -COOH, $-COOR_{10}$, $-CONH_2$, -NCO, -NCS, -COOX″, alkenyl, alkynyl, halide, tosylate, mesylate, tresylate, triflate and phenol, wherein R_{10} is alkyl, aryl, or alkylaryl and X″ is a halide.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to bioconjugates of manganese or iron complexes of nitrogen-containing fifteen-membered macrocyclic ligands which catalyze the conversion of superoxide into oxygen

and hydrogen peroxide. These complexes can be represented by the formula:



$\cdot(Z)_n$

wherein R, R′, R_1, R_1′, R_2, R_2′, R_3, R_3′, R_4, R_4′, R_5, R_5′, R_6, R_6′, R_7, R_7′, R_8, R_8′, R_9 and R_9′ independently represents hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkylalkyl, cycloalkylcycloalkyl, cycloalkenylalkyl, alkylcycloalkyl, alkenylcycloalkyl, alkylcycloalkenyl, alkenylcycloalkenyl, heterocyclic, aryl and aralkyl radicals and radicals attached to the α-carbon of α-amino acids; or R_1 or R'_1 and R_2 or R'_2, R_3 or R'_3 and R_4 or R'_4, R_5 or R'_5 and R_6 or R'_6, R_7 or R'_7 and R_8 or R'_8, and R_9 or R'_9 and R or R′ together with the carbon atoms to which they are attached independently form a saturated, partially saturated or unsaturated cyclic having 3 to 20 carbon atoms; or R or R′ and R_1 or R'_1, R_2 or R'_2 and R_3 or R'_3, R_4 or R'_4 and R_5 or R'_5, R_6 or R'_6 and R_7 or R'_7, and R_8 or R'_8 and R_9 or R'_9 together with the carbon atoms to which they are attached independently form a nitrogen containing heterocycle having 2 to 20 carbon atoms provided that when the nitrogen containing heterocycle is an aromatic heterocycle which does not contain a hydrogen attached to the nitrogen, the hydrogen attached to the nitrogen in said formula, which nitrogen is also in the

macrocycle and the R groups attached to the same carbon atoms of the macrocycle are absent; and combinations thereof; and wherein (1) one to five of the "R" groups are attached to biomolecules via a linker group, (2) one of X, Y and Z is attached to a biomolecule via a linker group, or (3) one to five of the "R" groups and one of X, Y and Z are attached to biomolecules via a linker group; and biomolecules are independently selected from the group consisting of steroids, carbohydrates, fatty acids, amino acids, peptides, proteins, antibodies, vitamins, lipids, phospholipids, phosphates, phosphonates, nucleic acids, enzyme substrates, enzyme inhibitors and enzyme receptor substrates and the linker group is derived from a substituent attached to the "R" group or X, Y and Z which is reactive with the biomolecule and is selected from the group consisting of $-NH_2$, $-NHR_{10}$, -SH, -OH, -COOH, $-COOR_{10}$, $-CONH_2$, -NCO, -NCS, -COOX″, alkenyl, alkynyl, halide, tosylate, mesylate, tresylate, triflate and phenol, wherein R_{10} is alkyl, aryl, or alkylaryl and X″ is a halide; and wherein M is Mn or Fe.

X, Y and Z represent suitable ligands or charge-neutralizing anions which are derived from any monodentate or polydentate coordinating ligand or ligand system or the corresponding anion thereof (for example benzoic acid or benzoate anion, phenol or phenoxide anion, alcohol or alkoxide anion). X, Y and Z are independently selected from the group consisting of halide, oxo, aquo, hydroxo, alcohol, phenol, dioxygen, peroxo, hydroperoxo, alkylperoxo, arylperoxo, ammonia, alkylamino, arylamino, heterocycloalkyl amino, heterocycloaryl amino, amine oxides, hydrazine, alkyl hydrazine, aryl hydrazine, nitric oxide, cyanide, cyanate, thiocyanate, isocyanate, isothiocyanate, alkyl nitrile, aryl nitrile, alkyl isonitrile, aryl isonitrile, nitrate, nitrite, azido, alkyl sulfonic

acid, aryl sulfonic acid, alkyl sulfoxide, aryl sulfoxide, alkyl aryl sulfoxide, alkyl sulfenic acid, aryl sulfenic acid, alkyl sulfinic acid, aryl sulfinic acid, alkyl thiol carboxylic acid, aryl thiol carboxylic acid, alkyl thiol thiocarboxylic acid, aryl thiol thiocarboxylic acid, alkyl carboxylic acid (such as acetic acid, trifluoroacetic acid, oxalic acid), aryl carboxylic acid (such as benzoic acid, phthalic acid), urea, alkyl urea, aryl urea, alkyl aryl urea, thiourea, alkyl thiourea, aryl thiourea,alkyl aryl thiourea, sulfate, sulfite, bisulfate, bisulfite, thiosulfate, thiosulfite, hydrosulfite, alkyl phosphine, aryl phosphine, alkyl phosphine oxide, aryl phosphine oxide, alkyl aryl phosphine oxide, alkyl phosphine sulfide, aryl phosphine sulfide, alkyl aryl phosphine sulfide, alkyl phosphonic acid, aryl phosphonic acid, alkyl phosphinic acid, aryl phosphinic acid, alkyl phosphinous acid, aryl phosphinous acid, phosphate, thiophosphate, phosphite, pyrophosphite, triphosphate, hydrogen phosphate, dihydrogen phosphate, alkyl guanidino, aryl guanidino, alkyl aryl guanidino, alkyl carbamate, aryl carbamate, alkyl aryl carbamate, alkyl thiocarbamate aryl thiocarbamate, alkyl aryl thiocarbamate, alkyl dithiocarbamate, aryl dithiocarbamate, alkyl aryl dithiocarbamate, bicarbonate, carbonate, perchlorate, chlorate, chlorite, hypochlorite, perbromate, bromate, bromite, hypobromite, tetrahalomanganate, tetrafluoroborate, hexafluorophosphate, hexafluoroantimonate, hypophosphite, iodate, periodate, metaborate, tetraaryl borate, tetra alkyl borate, tartrate, salicylate, succinate, citrate, ascorbate, saccharinate, amino acid, hydroxamic acid, thiotosylate, and anions of ion exchange resins, or systems where one or more of X,Y and Z are independently attached to one or more of the "R" groups, wherein n is 0 or 1. The preferred ligands from which X, Y and Z are

selected include halide, organic acid, nitrate and bicarbonate anions.

The linker groups, also termed herein "linker", are derived from the specified functional groups attached to the "R" groups or X, Y and Z, and function to link the biomolecule to the "R" groups or X, Y and Z. The functional groups are selected from the group consisting of $-NH_2$, $-NHR_{10}$, -SH, -OH, -COOH, $-COOR_{10}$, $-CONH_2$, -NCO, -NCS, -COOX″, alkenyl, alkynyl, halide, tosylate, mesylate, tresylate, triflate and phenol wherein R_{10} is alkyl, aryl, or alkaryl and X″ is a halide. Currently, the preferred alkenyl group is ethenyl and the preferred alkynyl group is ethynyl. The functional groups on the "R" groups or X, Y and Z are reactive with the biomolecule, i.e. reactive with a functional group on the steroids, carbohydrates, fatty acids, amino acids, peptides, proteins, antibodies, vitamins, lipids, phospholipids, phosphates, phosphonates, nucleic acids, enzyme substrates, enzyme inhibitors, enzyme receptor substrates and other targeting biomolecules of interest. When the functional group attached to the "R" groups or X, Y and Z reacts with the biomolecule, the functional group is modified and it is this derived functional group which is the linker. For example, when an $-NH_2$ functional group attached to an "R" group is reacted with a steroid such as in Example 1, the linker is -NH-. The exact structure of specific linker groups will be readily apparent to those of ordinary skill in the art and will depend on the specific functional group and biomolecule selected. The specific reaction conditions for reacting a functional group attached to "R" groups or X, Y and Z with a biomolecule will be readily apparent to those of ordinary skill in the art.

The functional group useful to form the linker, defined herein as a "linker precursor", may be present

on the "R" groups at the time the macrocycle is prepared or it may be added or modified after preparation of the macrocycle or manganese complex thereof. Similarly, the linker precursor can be present on an axial ligand, i.e. X, Y or Z, when the manganese or iron complex is prepared or an exchange reaction of the axial ligands is conducted to exchange the axial ligands present in the manganese or iron complex.

The macrocycle of the present invention can be complexed with manganese or iron either before or after conjugation with the targeting biomolecule depending on the specific biomolecule utilized. The conjugate of the macrocyclic complex and the targeting biomolecule is defined herein as a "bioconjugate".

Targeting of drugs is well known to those of ordinary skill in the art. See, for example, J. A. Katzenellenbogen et al, *Journal of Nuclear Medicine*, Vol. 33, No. 4, 1992, 558, and J.A. Katzenellenbogen et al, *Bioconjugate Chemistry*, **1991**, **2**, 353. Targeting agents are typically biomolecules. The biomolecules of the invention are biologically active molecules that are site specific, i.e. known to concentrate in the particular organ or tissue of interest. The biomolecules are selected to direct the tissue distribution of the bioconjugate via receptor binding, membrane association, membrane solubility, and the like. These biomolecules include, for example, steroids, carbohydrates (including monosaccharides, disaccharides and polysaccharides), fatty acids, amino acids, peptides, proteins, antibodies (including polyclonal and monoclonal and fragments thereof), vitamins, lipids, phospholipids, phosphates, phosphonates, nucleic acids, enzyme substrates, enzyme inhibitors and enzyme receptor substrates. The biomolecules also include those biomolecules which are combinations of the above biomolecules, such as a

combination of a steroid with a carbohydrate, e.g. digitonin.

The particular biomolecules which can be utilized to target a desired organ or tissue are known in the art or it will be readily apparent to those of ordinary skill in the art. The biomolecules of the invention are commercially available or can readily be prepared by one of ordinary skill in the art using conventional methods.

It is currently preferred that a maximum of one "R" group attached to the carbon atoms located between nitrogen atoms in the macrocycle has a biomolecule attached via a linker. In addition, the preferred compounds are those which have one to five, most preferably one to two, of the "R" groups attached to biomolecules and none of X, Y and Z attached to a biomolecule, or those which have one of X, Y and Z attached to a biomolecule and none of the "R" groups attached to a biomolecule.

Currently, the preferred compounds are those wherein at least one, more preferably at least two, of the "R" groups, in addition to the "R" groups which are attached to a biomolecule, represent alkyl, cycloalkyl alkyl and aralkyl radicals and the remaining "R" groups not attached to a biomolecule represent hydrogen, a saturated, partially saturated or unsaturated cyclic or a nitrogen containing heterocycle. Other preferred groups of compounds are those wherein at least one, preferably two, of R_1 or R'_1 and R_2 or R'_2, R_3 or R'_3 and R_4 or R'_4, R_5 or R'_5 and R_6 or R'_6, R_7 or R'_7 and R_8 or R'_8, and R_9 or R'_9 and R or R' together with the carbon atoms to which they are attached represent a saturated, partially saturated or unsaturated cyclic having 3 to 20 carbon atoms and the remaining "R" groups in addition to the "R" groups which are attached to a biomolecule via a linker are hydrogen, nitrogen containing heterocycles or alkyl groups, and those wherein at least one, preferably

two, of R or R′ and R_1 or R'_1, R_2 or R'_2 and R_3 or R'_3, R_4 or R'_4 and R_5 or R'_5, R_6 or R'_6, and R_7 or R'_7, and R_8 or R'_8 and R_9 or R'_9 together with the carbon atoms to which they are attached are bound to form a nitrogen containing heterocycle having 2 to 20 carbon atoms and the remaining "R" groups in addition to the "R" groups which are attached to a biomolecule via a linker are independently selected from hydrogen, saturated, partially saturated or unsaturated cyclics or alkyl groups.

As used herein, "R" groups means all of the R groups attached to the carbon atoms of the macrocycle, i.e., R, R′, R_1, R'_1, R_2, R'_2, R_3, R'_3, R_4, R'_4, R_5, R'_5, R_6, R'_6, R_7, R'_7, R_8, R'_8, R_9 and R'_9.

Another embodiment of the invention is a pharmaceutical composition in unit dosage form useful for dismutating superoxide comprising (a) a therapeutically or prophylactically effective amount of a complex as described above and (b) a nontoxic, pharmaceutically acceptable carrier, adjuvant or vehicle.

The commonly accepted mechanism of action of the manganese-based SOD enzymes involves the cycling of the manganese center between the two oxidation states (II,III). See J. V. Bannister, W. H. Bannister, and G. Rotilio, Crit. Rev. Biochem., 22, 111-180 (1987).

$$1)\quad Mn(II) + HO_2^{\cdot} \longrightarrow Mn(III) + HO_2^{\cdot}$$

$$2)\quad Mn(III) + O_2^{\dot{}} - \longrightarrow Mn(II) + O_2$$

The formal redox potentials for the $O_2/O_2^{\cdot-}$ and $HO_2^{\cdot}/H_2O_2$ couples at pH = 7 are -0.33 v and 0.87 v, respectively. See A. E. G. Cass, in Metalloproteins: Part 1, Metal Proteins with Redox Roles, ed. P. Harrison, P. 121. Verlag Chemie (Weinheim, GDR) (1985). For the above

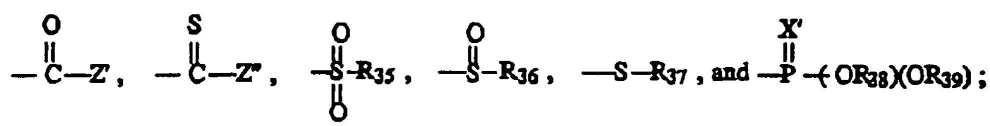
disclosed mechanism, these potentials require that a putative SOD catalyst be able to rapidly undergo oxidation state changes in the range of -0.33 v to 0.87 v.

The complexes derived from Mn(II) and the general class of C-substituted [15]aneN_5 ligands described herein have been characterized using cyclic voltammetry to measure their redox potential. The manganese-based C-substituted complexes described herein have reversible oxidations of about +0.7 v (SHE). Coulometry shows that this oxidation is a one-electron process; namely it is the oxidation of the Mn(II) complex to the Mn(III) complex. Thus, for these complexes to function as SOD catalysts, the Mn(III) oxidation state is involved in the catalytic cycle. This means that the Mn(III) complexes of all these ligands are equally competent as SOD catalysts, since it does not matter which form (Mn(II) or Mn(III)) is present when superoxide is present because superoxide will simply reduce Mn(III) to Mn(II) liberating oxygen.

The iron-based complexes of the invention are particularly useful due to the unexpectedly enhanced stability of the iron-based complexes compared to the corresponding manganese-based complexes. This enhanced stability could be important in oral administration and where targeted tissue has very low pH, e.g. ischemic tissue.

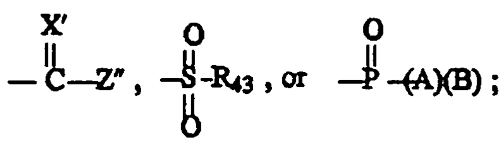
As utilized herein, the term "alkyl", alone or in combination, means a straight-chain or branched-chain alkyl radical containing from 1 to about 22 carbon atoms, preferably from about 1 to about 18 carbon atoms, and most preferably from about 1 to about 12 carbon atoms which optionally carries one or more substituents selected from (1) $-NR_{30}R_{31}$ wherein R_{30} and R_{31} are independently selected from hydrogen, alkyl, aryl or aralkyl; or R_{30} is hydrogen, alkyl, aryl or aralkyl and

R_{31} is selected from the group consisting of $-NR_{32}R_{33}$, -OH, $-OR_{34}$,

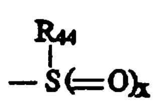


wherein R_{32} and R_{33} are independently hydrogen, alkyl, aryl or acyl, R_{34} is alkyl, aryl or alkaryl, Z' is hydrogen, alkyl, aryl, alkaryl, $-OR_{34}$, $-SR_{34}$ or $-NR_{40}R_{41}$ wherein R_{40} and R_{41} are independently selected from hydrogen, alkyl, aryl or alkaryl, Z'' is alkyl, aryl, alkaryl, $-OR_{34}$, $-SR_{34}$ or $-NR_{40}R_{41}$, R_{35} is alkyl, aryl, $-OR_{34}$, or $-NR_{40}R_{41}$, R_{36} is alkyl, aryl or $-NR_{40}R_{41}$. R_{37} is alkyl, aryl or alkaryl, X' is oxygen or sulfur, and R_{38} and R_{39} are independently selected from hydrogen, alkyl or aryl;

(2) $-SR_{42}$ wherein R_{42} is hydrogen, alkyl, aryl, alkaryl, $-SR_{34}$, $-NR_{32}R_{33}$,



wherein R_{43} is -OH, $-OR_{34}$ or $-NR_{32}R_{33}$, and A and B are independently $-OR_{34}$, $-SR_{34}$ or $-NR_{32}R_{33}$;



(3)

wherein x is 1 or 2, and R_{44} is halide, alkyl, aryl, alkaryl, -OH, $-OR_{34}$, $-SR_{34}$ or $-NR_{32}R_{33}$;

(4) $-OR_{45}$ wherein R_{45} is hydrogen, alkyl, aryl, alkaryl, $-NR_{32}R_{33}$,

$$-\overset{\overset{\displaystyle X'}{\|}}{C}-Z',\quad -\overset{\overset{\displaystyle R_{44}}{|}}{S}(=O)_x,\quad -\overset{\overset{\displaystyle O}{\|}}{P}-(D)(E),\ \text{or}\quad -\overset{\overset{\displaystyle O}{\|}}{P}-(R_{34})(OR_{34});$$

wherein D and E are independently $-OR_{34}$ or $-NR_{32}R_{33}$;

$$-\overset{\overset{\displaystyle X'}{\|}}{C}-R_{46}$$

(5)

wherein R_{46} is halide, -OH, -SH, $-OR_{34}$, $-SR_{34}$ or $-NR_{32}R_{33}$; or (6) amine oxides of the formula

$$-\underset{\underset{\displaystyle O^-}{|}}{\overset{+}{N}}R_{30}R_{31}$$

provided R_{30} and R_{31} are not hydrogen; or

$$-\overset{\overset{\displaystyle O}{\|}}{P}-(F)(G);$$

(7)

wherein F and G are independently -OH, -SH, $-OR_{34}$, $-SR_{34}$ or $-NR_{32}R_{33}$; or

(8) $-O-(-(CH_2)_a-O)_b-R_{10}$ wherein R_{10} is hydrogen or alkyl, and a and b an integers independently selected from 1 + 6; or

(9) halogen, cyano, nitro, or azido. Alkyl, aryl and alkaryl groups on the substituents of the above-defined alkyl groups may contain one additional substituent but are preferably unsubstituted. Examples of such radicals include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isoamyl, hexyl, octyl, nonyl, decyl,

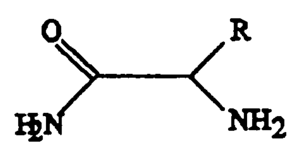
dodecyl, tetradecyl, hexadecyl, octadecyl and eicosyl. The term "alkenyl", alone or in combination, means an alkyl radical having one or more double bonds. Examples of such alkenyl radicals include, but are not limited to, ethenyl, propenyl, 1-butenyl, cis-2-butenyl, trans-2-butenyl, iso-butylenyl, cis-2-pentenyl, trans-2-pentenyl, 3-methyl-1-butenyl, 2,3-dimethyl-2-butenyl, 1-pentenyl, 1-hexenyl, 1-octenyl, decenyl, dodecenyl, tetradecenyl, hexadecenyl, cis- and trans-9-octadecenyl, 1,3-pentadienyl, 2,4-pentadienyl, 2,3-pentadienyl, 1,3-hexadienyl, 2,4-hexadienyl, 5,8,11,14-eicosatetraenyl, and 9,12,15-octadecatrienyl. The term "alkynyl", alone or in combination, means an alkyl radical having one or more triple bonds. Examples of such alkynyl groups include, but are not limited to, ethynyl, propynyl (propargyl), 1-butynyl, 1-octynyl, 9-octadecynyl, 1,3-pentadiynyl, 2,4-pentadiynyl, 1,3-hexadiynyl, and 2,4-hexadiynyl. The term "cycloalkyl", alone or in combination means a cycloalkyl radical containing from 3 to about 10, preferably from 3 to about 8, and most preferably from 3 to about 6, carbon atoms. Examples of such cycloalkyl radicals include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, and perhydronaphthyl. The term "cycloalkylalkyl" means an alkyl radical as defined above which is substituted by a cycloalkyl radical as defined above. Examples of cycloalkylalkyl radicals include, but are not limited to, cyclohexylmethyl, cyclopentylmethyl, (4-isopropylcyclohexyl)methyl, (4-t-butyl-cyclohexyl)methyl, 3-cyclohexylpropyl, 2-cyclo-hexylmethylpentyl, 3-cyclopentylmethylhexyl, 1-(4-neopentylcyclohexyl)methylhexyl, and 1-(4-isopropylcyclohexyl)methylheptyl. The term "cycloalkylcycloalkyl" means a cycloalkyl radical as

defined above which is substituted by another cycloalkyl radical as defined above. Examples of cycloalkylcycloalkyl radicals include, but are not limited to, cyclohexylcyclopentyl and cyclohexylcyclohexyl. The term "cycloalkenyl", alone or in combination, means a cycloalkyl radical having one or more double bonds. Examples of cycloalkenyl radicals include, but are not limited to, cyclopentenyl, cyclohexenyl, cyclooctenyl, cyclopentadienyl, cyclohexadienyl and cyclooctadienyl. The term "cycloalkenylalkyl" means an alkyl radical as defined above which is substituted by a cycloalkenyl radical as defined above. Examples of cycloalkenylalkyl radicals include, but are not limited to, 2-cyclohexen-1-ylmethyl, 1-cyclopenten-1-ylmethyl, 2-(1-cyclohexen-1-yl)ethyl, 3-(1-cyclopenten-1-yl)propyl, 1-(1-cyclohexen-1-ylmethyl)pentyl, 1-(1-cyclopenten-1-yl)hexyl, 6-(1-cyclohexen-1-yl)hexyl, 1-(1-cyclopenten-1-yl)nonyl and 1-(1-cyclohexen-1-yl)nonyl. The terms "alkylcycloalkyl" and "alkenylcycloalkyl" mean a cycloalkyl radical as defined above which is substituted by an alkyl or alkenyl radical as defined above. Examples of alkylcycloalkyl and alkenylcycloalkyl radicals include, but are not limited to, 2-ethylcyclobutyl, 1-methylcyclopentyl, 1-hexylcyclopentyl, 1-methylcyclohexyl, 1-(9-octadecenyl)cyclopentyl and 1-(9-octadecenyl)cyclohexyl. The terms "alkylcycloalkenyl" and "alkenylcycloalkenyl" means a cycloalkenyl radical as defined above which is substituted by an alkyl or alkenyl radical as defined above. Examples of alkylcycloalkenyl and alk nylcycloalkenyl radicals include, but are not limited to, 1-methyl-2-cyclopentenyl, 1-hexyl-2-cyclopentenyl, 1-ethyl-2-cyclohexenyl,

1-butyl-2-cyclohexenyl, 1-(9-octadecenyl)-2-cyclohexenyl and 1-(2-pentenyl)-2-cyclohexenyl. The term "aryl", alone or in combination, means a phenyl or naphthyl radical which optionally carries one or more substituents selected from alkyl, cycloalkyl, cycloalkenyl, aryl, heterocycle, alkoxyaryl, alkaryl, alkoxy, halogen, hydroxy, amine, cyano, nitro, alkylthio, phenoxy, ether, trifluoromethyl and the like, such as phenyl, p-tolyl, 4-methoxyphenyl, 4-(tert-butoxy)phenyl, 4-fluorophenyl, 4-chlorophenyl, 4-hydroxyphenyl, 1-naphthyl, 2-naphthyl, and the like. The term "aralkyl", alone or in combination, means an alkyl or cycloalkyl radical as defined above in which one hydrogen atom is replaced by an aryl radical as defined above, such as benzyl, 2-phenylethyl, and the like. The term "heterocyclic" means ring structures containing at least one other kind of atom, in addition to carbon, in the ring. The most common of the other kinds of atoms include nitrogen, oxygen and sulfur. Examples of heterocyclics include, but are not limited to, pyrrolidinyl, piperidyl, imidazolidinyl, tetrahydrofuryl, tetrahydrothienyl, furyl, thienyl, pyridyl, quinolyl, isoquinolyl, pyridazinyl, pyrazinyl, indolyl, imidazolyl, oxazolyl, thiazolyl, pyrazolyl, pyridinyl, benzoxadiazolyl, benzothiadiazolyl, triazolyl and tetrazolyl groups. The term "saturated, partially saturated or unsaturated cyclic" means fused ring structures in which 2 carbons of the ring are also part of the fifteen-membered macrocyclic ligand. The ring structure can contain 3 to 20 carbon atoms, preferably 5 to 10 carbon atoms, and can also contain one or more other kinds of atoms in addition to carbon. The most common of the other kinds of atoms include nitrogen, oxygen and sulfur. The ring structure can also contain more than one ring. The term "saturated, partially saturated or unsaturated ring structure" means a ring

structure in which one carbon of the ring is also part of the fifteen-membered macrocyclic ligand. The ring structure can contain 3 to 20, preferably 5 to 10, carbon atoms and can also contain nitrogen, oxygen and/or sulfur atoms. The term "nitrogen containing heterocycle" means ring structures in which 2 carbons and a nitrogen of the ring are also part of the fifteen-membered macrocyclic ligand. The ring structure can contain 2 to 20, preferably 4 to 10, carbon atoms, can be partially or fully unsaturated or saturated and can also contain nitrogen, oxygen and/or sulfur atoms in the portion of the ring which is not also part of the fifteen-membered macrocyclic ligand. The term "organic acid anion" refers to carboxylic acid anions having from about 1 to about 18 carbon atoms. The term "halide" means chloride or bromide.

The macrocyclic ligands useful in the complexes of the present invention can be prepared according to the general procedure shown in Scheme A set forth below. Thus, an amino acid amide, which is the corresponding amide derivative of a naturally or non-naturally occurring α-amino acid, is reduced to form the corresponding substituted ethylenediamine. Such amino acid amide can be the amide derivative of any one of many well known amino acids. Preferred amino acid amides are those represented by the formula:



wherein R is derived from the D or L forms of the amino acids Alanine, Aspartic acid, Arginine, Asparagine, Cystein , Glycine, Glutamic acid, Glutamine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Proline,

Phenylalanine, Serine, Tryptophan, Threonine, Tyrosine, Valine and/or the R groups of unnatural α-amino acids such as alkyl, ethyl, butyl, tert-butyl, cycloalkyl, phenyl, alkenyl, allyl, alkynyl, aryl, heteroaryl, polycycloalkyl, polycycloaryl, polycycloheteroaryl, imines, aminoalkyl, hydroxyalkyl, hydroxyl, phenol, amine oxides, thioalkyl, carboalkoxyalkyl, carboxylic acids and their derivatives, keto, ether, aldehyde, amine, nitrile, halo, thiol, sulfoxide, sulfone, sulfonic acid, sulfide, disulfide, phosphonic acid, phosphinic acid, phosphine oxides, sulfonamides, amides, amino acids, peptides, proteins, carbohydrates, nucleic acids, fatty acids, lipids, nitro, hydroxylamines, hydroxamic acids, thiocarbonyls, borates, boranes, boraza, silyl, siloxy, silaza, and combinations thereof. Most preferred are those wherein R represents hydrogen, alkyl, cycloalkylalkyl, and aralkyl radicals. The diamine is then tosylated to produce the di-N-tosyl derivative which is reacted with a di-O-tosylated tris-N-tosylated triazaalkane diol to produce the corresponding substituted N-pentatosylpentaazacycloalkane. The tosyl groups are then removed and the resulting compound is reacted with a manganese(II) or iron (III) compound under essentially anhydrous and anaerobic conditions to form the corresponding substituted manganese(II) or iron (III) pentaazacycloalkane complex. When the ligands or charge-neutralizing anions, i.e. X, Y and Z, are anions or ligands that cannot be introduced directly from the manganese or iron compound, the complex with those anions or ligands can be formed by conducting an exchange reaction with a complex that has been prepared by reacting the macrocycle with a manganese or iron compound.

The complexes of the present invention, wherein R_9, and R_2 are alkyl, and R_3, R'_3, R_4, R'_4, R_5, R'_5, R_6, R'_6,

R_7, R'_7, R_8 and R'_8 can be alkyl, arylalkyl or cycloalkylalkyl and R or R' and R_1 or R'_1 together with the carbon atoms they are attached to are bound to form a nitrogen containing heterocycle, can also be prepared according to the general procedure shown in Scheme B set forth below utilizing methods known in the art for preparing the manganese(II) or iron (III) pentaazabicyclo[12.3.1]octadecapentaene complex precursor. See, for example, Alexander et al., Inorg. Nucl. Chem. Lett., 6, 445 (1970). Thus a 2,6-diketopyridine is condensed with triethylene tetraamine in the presence of a manganese(II) or iron (III) compound to produce the manganese(II) or iron (III) pentaazabicyclo[12.3.1]octadecapentaene complex. The manganese(II) or iron (III) pentaazabicyclo[12.3.1]octadecapentaene complex is hydrogenated with platinum oxide at a pressure of 10-1000 psi to give the corresponding manganese(II) or iron (III) pentaazabicyclo[12.3.1]octadecatriene complex.

The macrocyclic ligands useful in the complexes of the present invention can also be prepared by the diacid dichloride route shown in Scheme C set forth below. Thus, a triazaalkane is tosylated in a suitable solvent system to produce the corresponding tris (N-tosyl) derivative. Such a derivative is treated with a suitable base to produce the corresponding disulfonamide anion. The disulfonamide anion is dialkylated with a suitable electrophile to produce a derivative of a dicarboxylic acid. This derivative of a dicarboxylic acid is treated to produce the dicarboxylic acid, which is then treated with a suitable reagent to form the diacid dichloride. The desired vicinal diamine is obtained in any of several ways. One way which is useful is the preparation from an aldehyde by reaction with cyanid in the presence of ammonium chloride followed by treatment with acid to produce the alpha

ammonium nitrile. The latter compound is reduced in the presence of acid and then treated with a suitable base to produce the vicinal diamine. Condensation of the diacid dichloride with the vicinal diamine in the presence of a suitable base forms the tris(tosyl)diamide macrocycle. The tosyl groups are removed and the amides are reduced and the resulting compound is reacted with a manganese (II) or iron (III) compound under essentially anhydrous and anaerobic conditions to form the corresponding substituted pentaazacycloalkane manganese (II) or iron (III) complex.

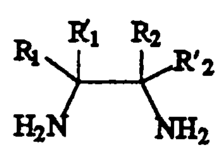
The vicinal diamines have been prepared by the route shown (known as the Strecker synthesis) and vicinal diamines were purchased when commercially available. Any method of vicinal diamine preparation could be used.

The macrocyclic ligands useful in the complexes of the present invention can also be prepared by the pyridine diamide route shown in Scheme D as set forth below. Thus, a polyamine, such as a tetraaza compound, containing two primary amines is condensed with dimethyl 2,6-pyridine dicarboxylate by heating in an appropriate solvent, e.g., methanol, to produce a macrocycle incorporating the pyridine ring as the 2,6-dicarboxamide. The pyridine ring in the macrocycle is reduced to the corresponding piperidine ring in the macrocycle, and then the diamides are reduced and the resulting compound is reacted with a manganese (II) or iron (III) compound under essentially anhydrous and anaerobic conditions to form the corresponding substituted pentaazacycloalkane manganese (II) or iron (III) complex.

The macrocyclic ligands useful in the complexes of the present invention can also be prepared by the bis(haloacetamide) route shown in Scheme E set forth below. Thus a triazaalkane is tosylated in a suitable solvent system to produce the corresponding tris

(N-tosyl) derivative. Such a derivative is treated with a suitable base to produce the corresponding disulfonamide anion. A bis(haloacetamide), e.g., a bis(chloroacetamide), of a vicinal diamine is prepared by reaction of the diamine with an excess of haloacetyl halide, e.g., chloroacetyl chloride, in the presence of a base. The disulfonamide anion of the tris(N-tosyl) triazaalkane is then reacted with the bis(chloroacetamide) of the diamine to produce the substituted tris(N-tosyl)diamide macrocycle. The tosyl groups are removed and the amides are reduced and the resulting compound is reacted with a manganese (II) or iron (III) compound under essentially anhydrous and anaerobic conditions to form the corresponding substituted pentaazacycloalkane manganese (II) or iron (III) complex.

The macrocyclic ligands useful in the complexes of the present invention, wherein R_1, R'_1, R_2, R'_2 are derived from a diamino starting material and R_5, R'_5, R_7, R'_7 and R_9, R'_9 can be H or any functionality previously described, can be prepared according to the pseudo-peptide method shown in Scheme F set forth below. A substituted 1,2-diaminoethane represented by the formula



, wherein R_1, R'_1, R_2 and R'_2 are the substituents on adjacent carbon atoms in the product macrocyclic ligand as set forth above, can be used in this method in combination with any amino acids. The diamine can be produced by any conventional method known to those

skilled in the art. The R groups in the macrocycle derived from substituents on the α-carbon of α-amino acids, i.e. R_5, R'_5, R_7, R'_7, R_9 and R'_9, could be derived from the D or L forms of the amino acids Alanine, Aspartic acid, Arginine, Asparagine, Cysteine, Glycine, Glutamic acid, Glutamine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Proline, Phenylalanine, Serine, Tryptophan, Threonine, Tyrosine, Valine and/or the R groups of unnatural α-amino acids such as alkyl, ethyl, butyl, tert-butyl, cycloalkyl, phenyl, alkenyl, allyl, alkynyl, aryl, heteroaryl, polycycloalkyl, polycycloaryl, polycycloheteroaryl, imines, aminoalkyl, hydroxyalkyl, hydroxyl, phenol, amine oxides, thioalkyl, carboalkoxyalkyl, carboxylic acids and their derivatives, keto, ether, aldehyde, amine, nitrile, halo, thiol, sulfoxide, sulfone, sulfonic acid, sulfide, disulfide, phosphonic acid, phosphinic acid, phosphine oxides, sulfonamides, amides, amino acids, peptides, proteins, carbohydrates, nucleic acids, fatty acids, lipids, nitro, hydroxylamines, hydroxamic acids, thiocarbonyls, borates, boranes, boraza, silyl, siloxy, silaza, and combinations thereof. As an example 1,8-dihydroxy, 4,5-diaminooctane is monotosylated and reacted with Boc anhydride to afford the differentiated N-Boc, N-tosyl derivative. The sulfonamide was alkylated with methyl bromoacetate using sodium hydride as the base and saponified to the free acid. The diamine containing N-tosylglycine serves as a dipeptide surrogate in standard solution-phase peptide synthesis. Thus, coupling with a functionalized amino acid ester affords the corresponding pseudo-tripeptide. Two sequential TFA cleavage-couplings affords the pseudo-pentapeptide which can be N- and C-terminus deprotected in one step using HCl/AcOH. DPPA mediated cyclization followed by $LiAlH_4$ or Borane reduction affords the corresponding macrocylic ligand. This ligand system is reacted with a manganese (II) or iron (III) compound,

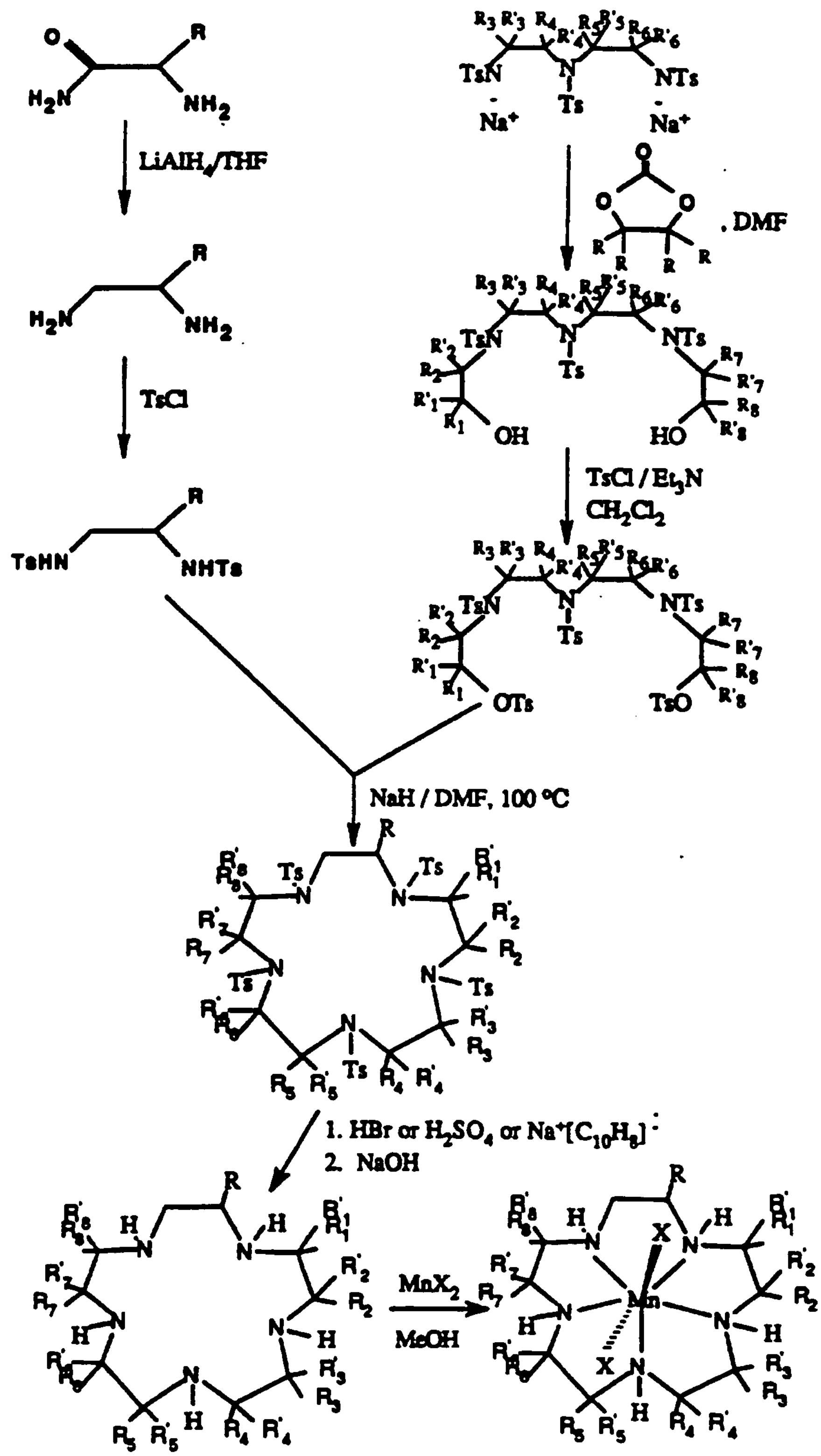
such as manganese (II) chloride or iron (III) chloride, under essentially anaerobic conditions to form the corresponding functionalized manganese (II) or iron (III) pentaazacycloalkane complex. When the ligands or charge-neutralizing anions, i.e. X, Y and Z, are anions or ligands that cannot be introduced directly from the manganese or iron compound, the complex with those anions or ligands can be formed by conducting an exchange reaction with a complex that has been prepared by reacting the macrocycle with a manganese or iron compound.

The macrocyclic ligands useful in the complexes of the present invention, wherein R_1, R'_1, R_3, R'_3, R_5, R'_5, R_7, R'_7, R_9 and R'_9 can be H or any functionality as previously described, can be prepared according to the general peptide method shown in Scheme G set forth below. The R groups in the macrocycle derived from substitutents on the α-carbon of α-amino acids, i.e. R_1, R'_1, R_3, R'_3, R_5, R'_5, R_7, R'_7, R_9 and R'_9, are defined above in Scheme F. The procedure for preparing the cyclic peptide precursors from the corresponding linear peptides are the same or significant modifications of methods known in the art. See, for example, Veber, D.F. et al., J. Org. Chem., 44, 3101 (1979). The general method outlined in Scheme G below is an example utilizing the sequential solution-phase preparation of the functionalized linear pentapeptide from N-terminus to C-terminus. Alternatively, the reaction sequence to prepare the linear pentapeptide can be carried out by solid-phase preparation utilizing methods known in the art. The reaction sequence could be conducted from C-terminus to N-terminus and by convergent approaches such as the coupling of di- and tri-peptides as needed. Thus a Boc-protected amino acid is coupled with an amino acid ester using standard peptide coupling reagents. The new Boc-dipeptide ester is then saponified to the free

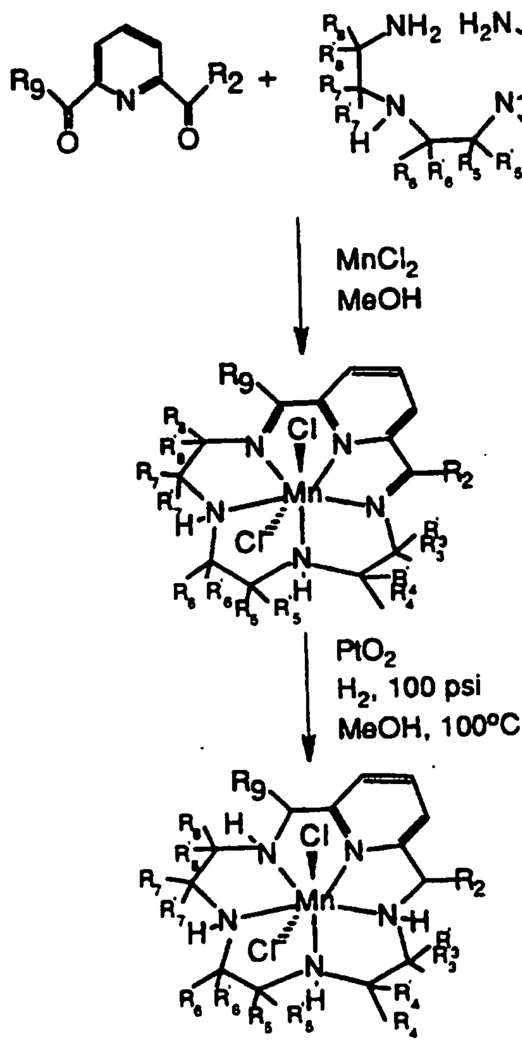
acid which is coupled again to another amino acid ester. The resulting Boc-tri-peptide ester is again saponified and this method is continued until the Boc-protected pentapeptide free acid has been prepared. The Boc protecting group is removed under standard conditions and the resulting pentapeptide or salt thereof is converted to the cyclic pentapeptide. The cyclic pentapeptide is then reduced to the pentaazacyclopentadecane with lithium aluminum hydride or borane. The final ligand is then reacted with a manganese (II) or iron (III) compound under essentially anaerobic conditions to form the corresponding manganese (II) or iron (III) pentaazacyclopentadecane complex. When the ligands or charge-neutralizing anions, e.g. X,Y and Z, are anions or ligands that cannot be introduced directly from the manganese or iron compound, the complex with those anions or ligands can be formed by conducting an exchange reaction with a complex that has been prepared by reacting the macrocycle with a manganese or iron compound.

The following schemes are depicted for preparing the manganese complexes of the invention. The iron complexes of the invention can be prepared by substituting an iron compound for the manganese compound used.

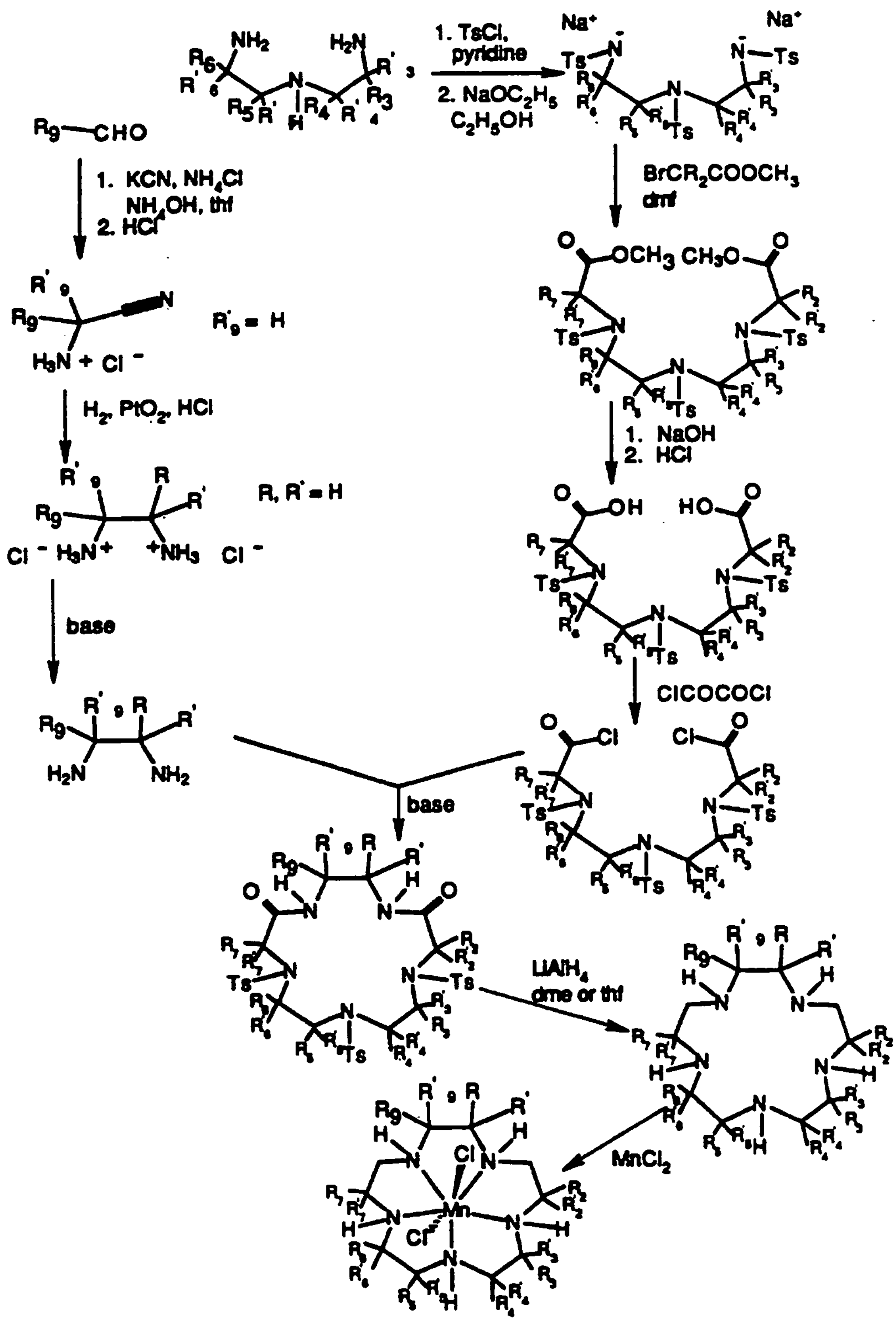
SCHEME A



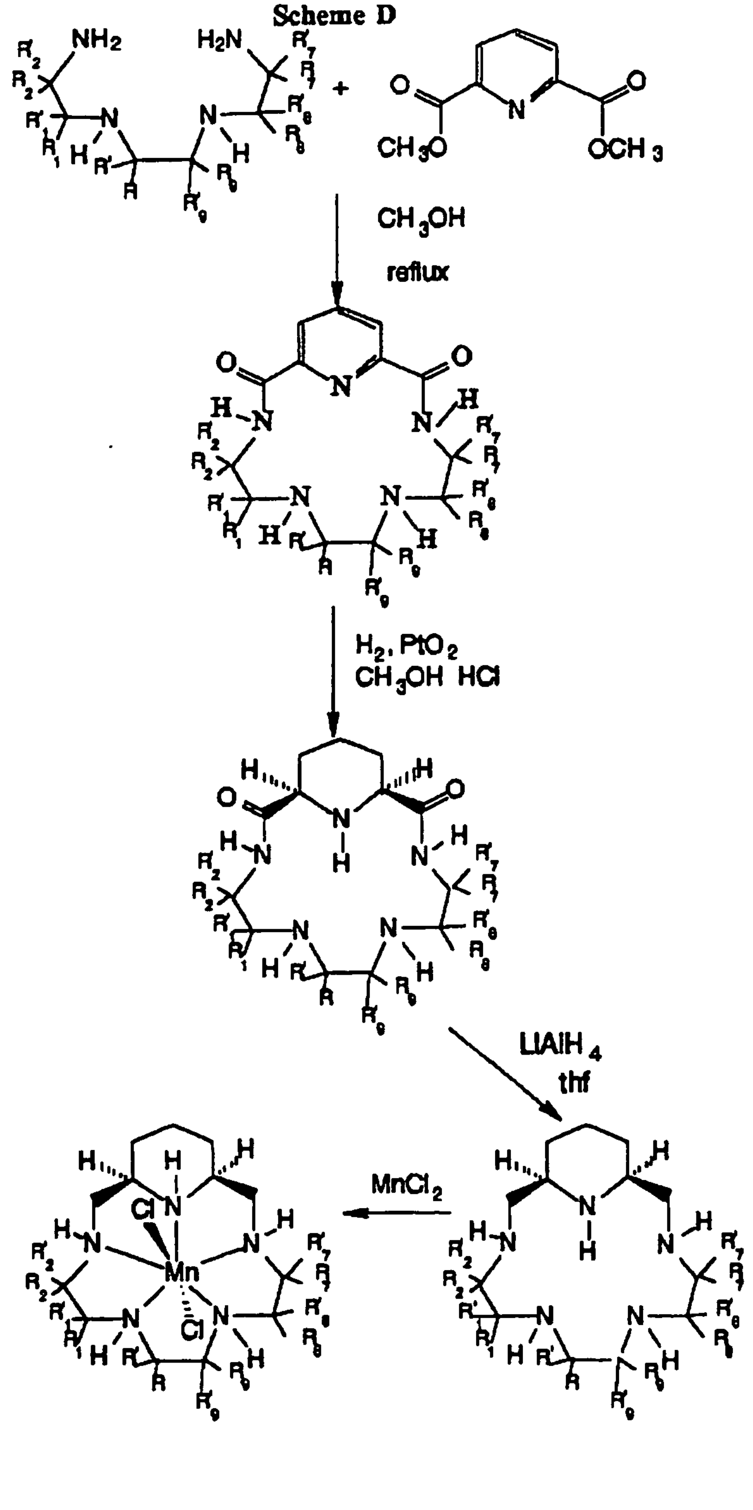
SCHEME B



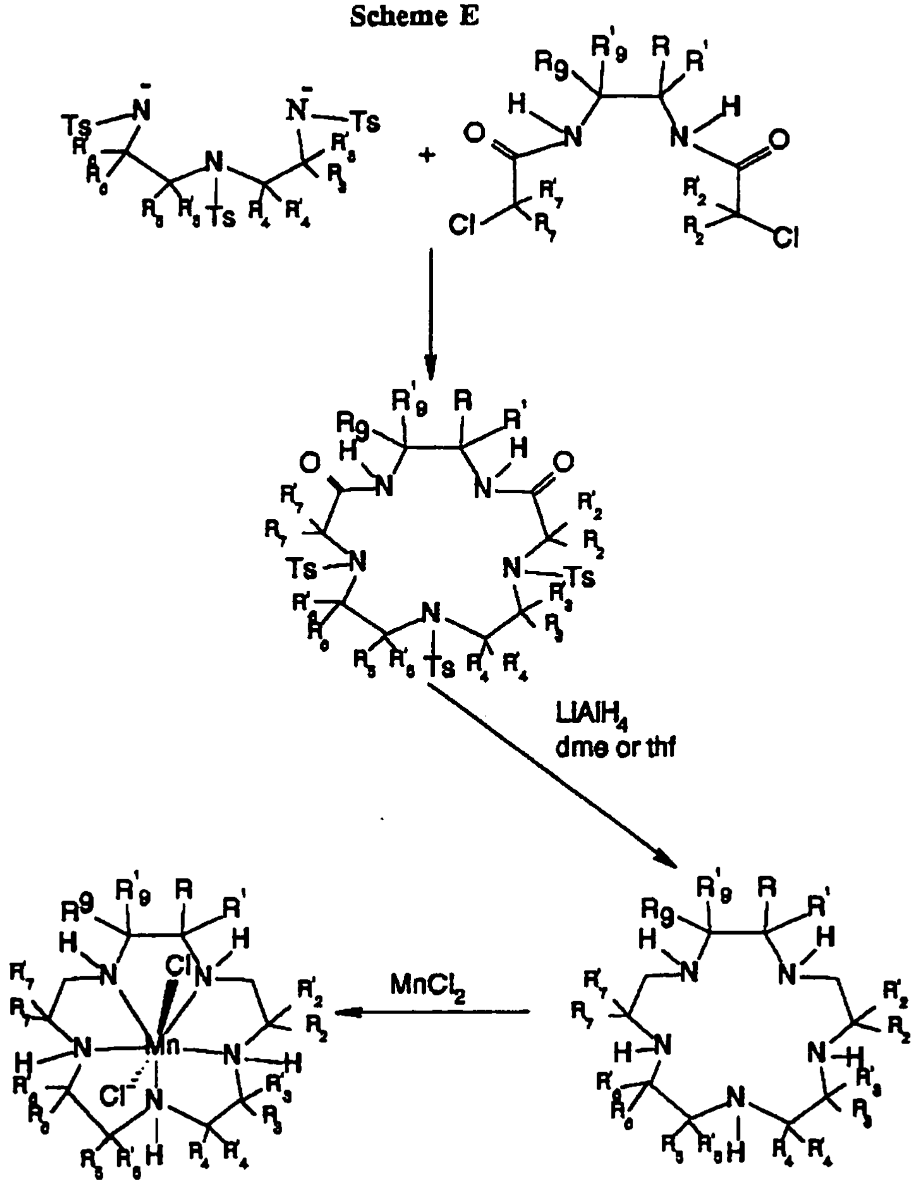
SCHEME C


1. TsCl, pyridine
2. NaOC$_2$H$_5$ C$_2$H$_5$OH
BrCR$_2$COOCH$_3$ dmf
1. NaOH
2. HCl
ClCOCOCl
1. KCN, NH$_4$Cl NH$_4$OH, thf
2. HCl
H$_2$, PtO$_2$, HCl
base
R'$_9$ = H
R, R' = H
LiAlH$_4$ dme or thf
MnCl$_2$

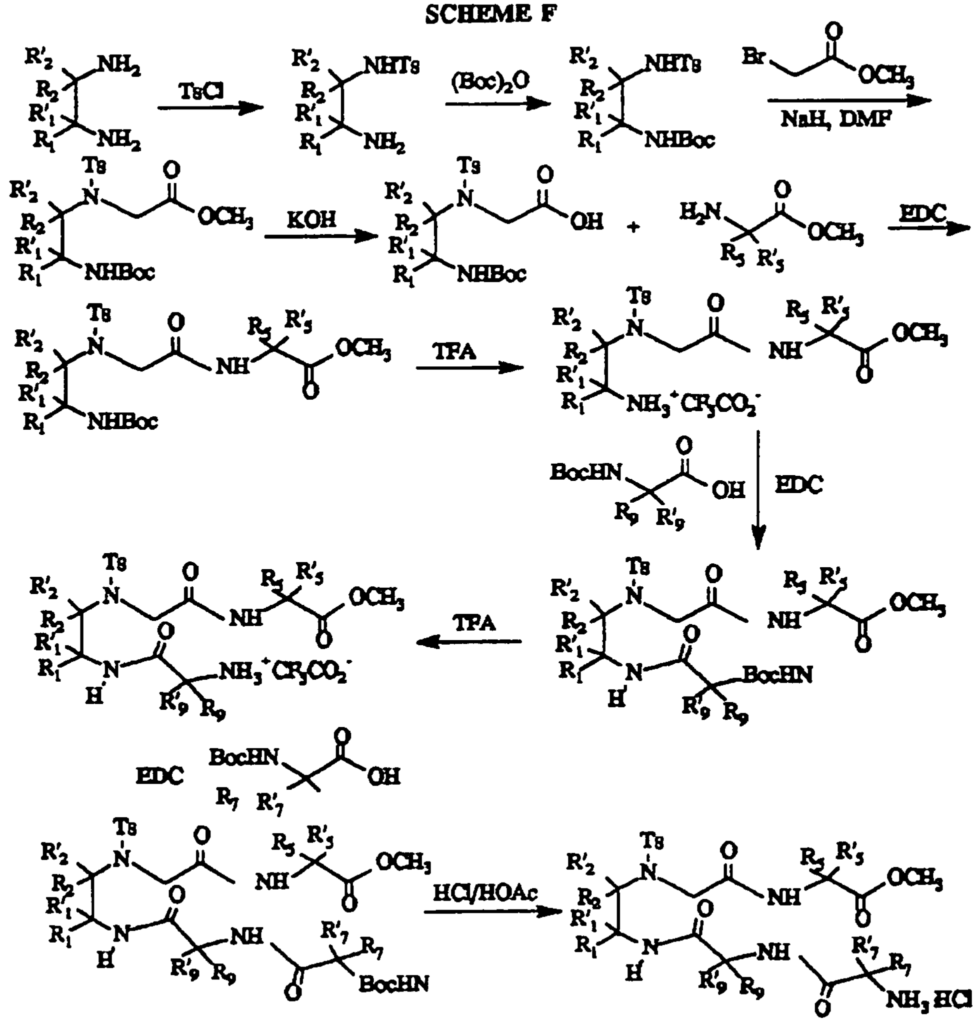
Scheme D


NH2
H2N
N
H
CH3O
OCH3
O
CH3OH
reflux
H2, PtO2
CH3OH HCl
LiAlH4
thf
MnCl2
Cl
Mn

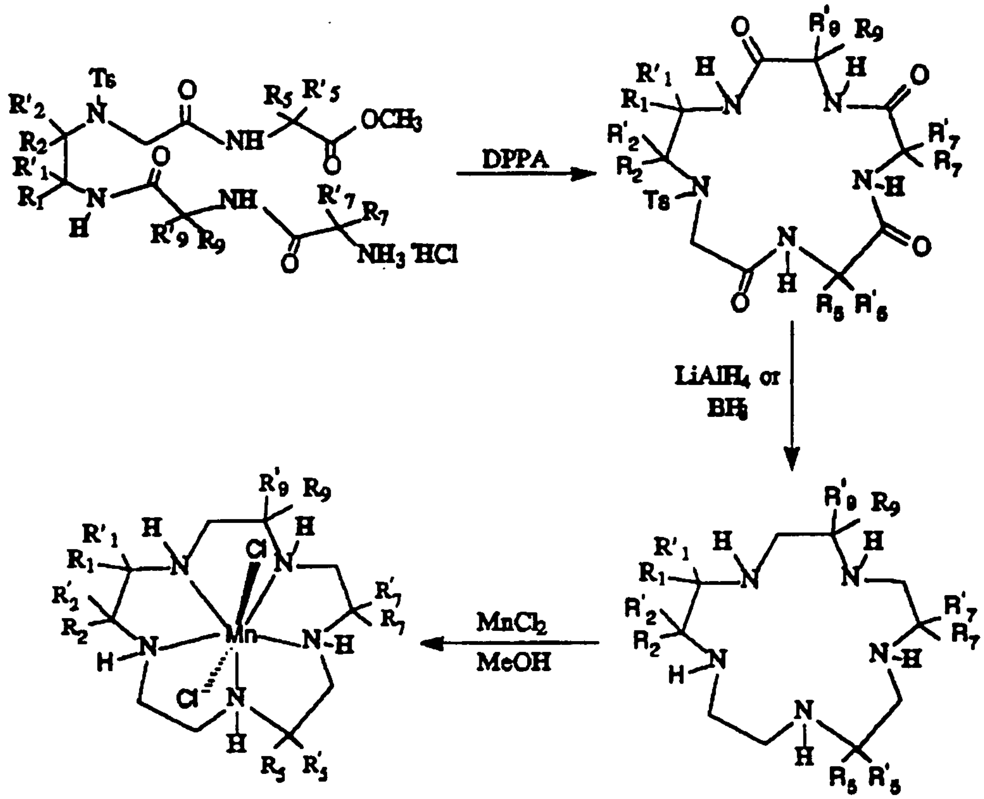
Scheme E



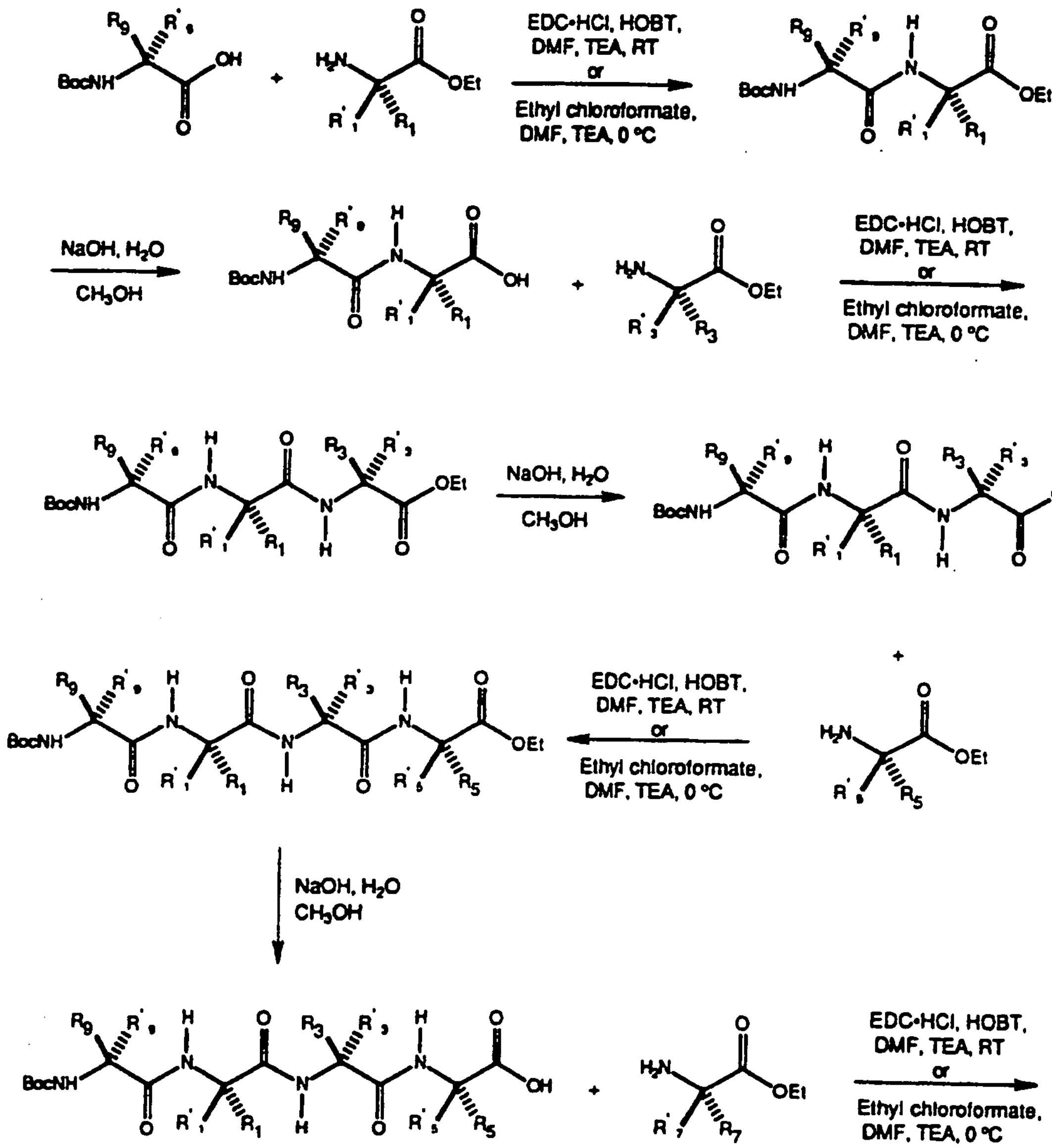
SCHEME F



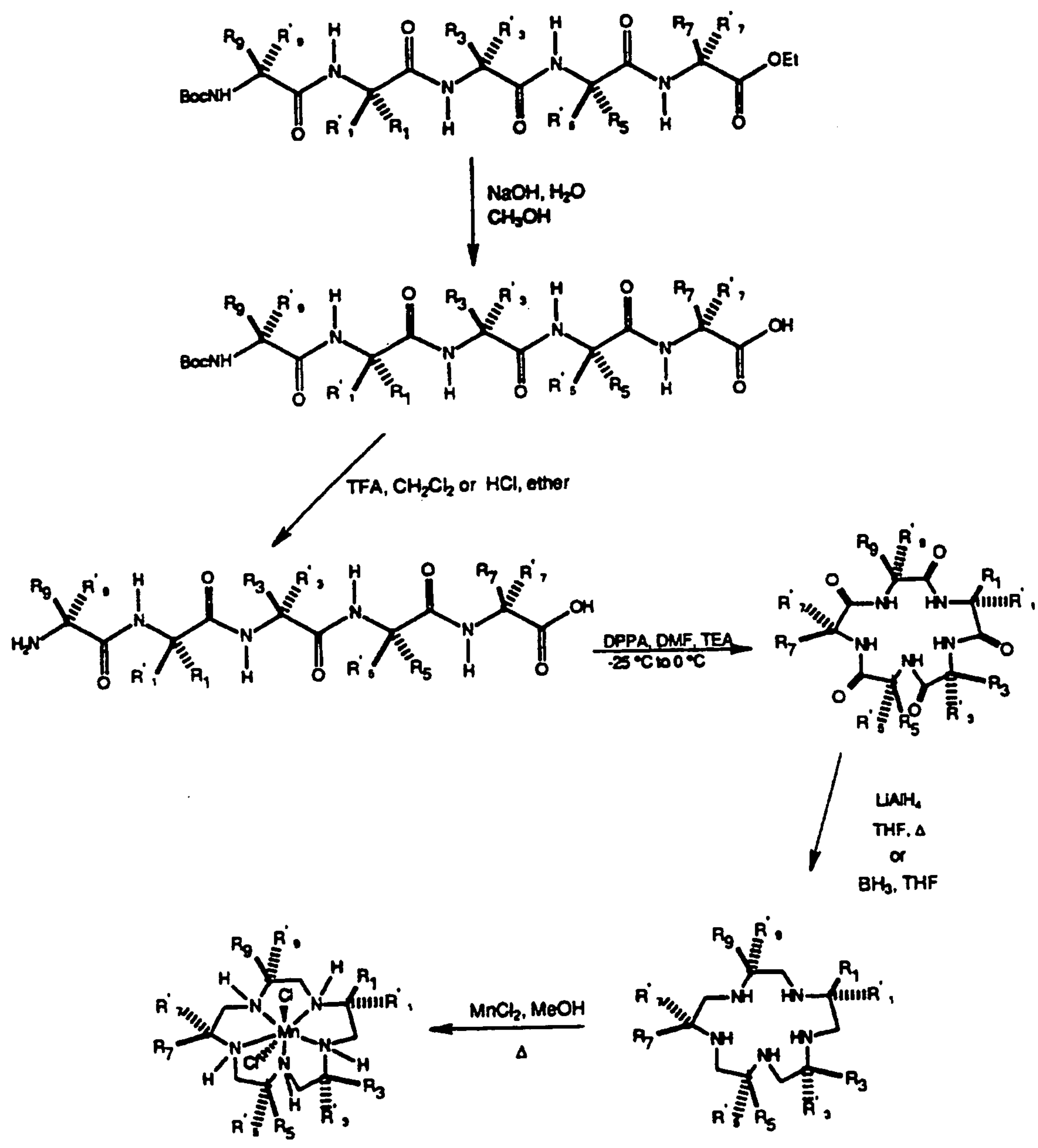
SCHEME F (cont.)



SCHEME G


BocNH
OH
O
+
H_2N
OEt
EDC•HCl, HOBT,
DMF, TEA, RT
or
Ethyl chloroformate,
DMF, TEA, 0 °C
NaOH, H_2O
CH_3OH
R_9
R_1
R_3
R_5
R_7

SCHEME G (cont.)



The pentaazamacrocycles of the present invention can possess one or more asymmetric carbon atoms and are thus capable of existing in the form of optical isomers as well as in the form of racemic or nonracemic mixtures thereof. The optical isomers can be obtained by resolution of the racemic mixtures according to conventional processes, for example by formation of diastereoisomeric salts by treatment with an optically active acid. Examples of appropriate acids are tartaric, diacetyltartaric, dibenzoyltartaric, ditoluoyltartaric and camphorsulfonic acid and then separation of the mixture of diastereoisomers by crystallization followed by liberation of the optically active bases from these salts. A different process for separation of optical isomers involves the use of a chiral chromatography column optimally chosen to maximize the separation of the enantiomers. Still another available method involves synthesis of covalent diastereoisomeric molecules by reacting one or more secondary amine group(s) of the compounds of the invention with an optically pure acid in an activated form or an optically pure isocyanate. The synthesized diastereoisomers can be separated by conventional means such as chromatography, distillation, crystallization or sublimation, and then hydrolyzed to deliver the enantiomerically pure ligand. The optically active compounds of the invention can likewise be obtained by utilizing optically active starting materials, such as natural amino acids.

The compounds or complexes of the present invention are novel and can be utilized to treat numerous inflammatory disease states and disorders. For example, reperfusion injury to an ischemic organ, e.g., reperfusion injury to the ischemic myocardium, surgically-induced ischemia, inflammatory bowel disease, rheumatoid arthritis, osteoarthritis, psoriasis, organ transplant rejections, radiation-induced injury, oxidant-

induced tissue injuries and damage, atherosclerosis, thrombosis, platelet aggregation, stroke, acute pancreatitis, insulin-dependent diabetes mellitus, disseminated intravascular coagulation, fatty embolism, adult and infantile respiratory distress, metastasis and carcinogenesis.

Activity of the compounds or complexes of the present invention for catalyzing the dismutation of superoxide can be demonstrated using the stopped-flow kinetic analysis technique as described in Riley, D.P., Rivers, W.J. and Weiss, R.H., "Stopped-Flow Kinetic Analysis for Monitoring Superoxide Decay in Aqueous Systems," Anal. Biochem., 196, 344-349 (1991), which is incorporated by reference herein. Stopped-flow kinetic analysis is an accurate and direct method for quantitatively monitoring the decay rates of superoxide in water. The stopped-flow kinetic analysis is suitable for screening compounds for SOD activity and catalytic activity of the compounds or complexes of the present invention for dismutating superoxide, as shown by stopped-flow analysis, correlate to treating the above disease states and disorders.

Total daily dose administered to a host in single or divided doses may be in amounts, for example, from about 1 to about 100 mg/kg body weight daily and more usually about 3 to 30 mg/kg. Unit dosage compositions may contain such amounts of submultiples thereof to make up the daily dose.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

The dosage regimen for treating a disease condition with the compounds and/or compositions of this invention is selected in accordance with a variety of factors, including the type, age, weight, sex, diet and

medical condition of the patient, the severity of the disease, the route of administration, pharmacological considerations such as the activity, efficacy, pharmacokinetic and toxicology profiles of the particular compound employed, whether a drug delivery system is utilized and whether the compound is administered as part of a drug combination. Thus, the dosage regimen actually employed may vary widely and therefore may deviate from the preferred dosage regimen set forth above.

The compounds of the present invention may be administered orally, parenterally, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired. Topical administration may also involve the use of transdermal administration such as transdermal patches or iontophoresis devices. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection, or infusion techniques.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Suppositories for rectal administration of the drug can be prepared by mixing the drug with a suitable nonirritating excipient such as cocoa butter and polyethylene glycols which are solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum and release the drug.

Solid dosage forms for oral administration may include capsules, tablets, pills, powders, granules and gels. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose lactose or starch. Such dosage forms may also comprise, as in normal practice, additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

While the compounds of the invention can be administered as the sole active pharmaceutical agent, they can also be used in combination with one or more compounds which are known to be effective against the specific disease state that one is targeting for treatment.

The compounds or complexes of the invention can also be utilized as MRI contrast agents. A discussion of the use of contrast agents in MRI can be found in patent application Serial No. 08/397,469, which is incorporated by reference herein.

Contemplated equivalents of the general formulas

set forth above for the compounds and derivatives as well as the intermediates are compounds otherwise corresponding thereto and having the same general properties such as tautomers of the compounds and such as wherein one or more of the various R groups are simple variations of the substituents as defined therein, e.g., wherein R is a higher alkyl group than that indicated, or where the tosyl groups are other nitrogen or oxygen protecting groups or wherein the O-tosyl is a halide. Anions having a charge other than 1, e.g., carbonate, phosphate, and hydrogen phosphate, can be used instead of anions having a charge of 1, so long as they do not adversely affect the overall activity of the complex. However, using anions having a charge other than 1 will result in a slight modification of the general formula for the complex set forth above. In addition, where a substituent is designated as, or can be, a hydrogen, the exact chemical nature of a substituent which is other than hydrogen at that position, e.g., a hydrocarbyl radical or a halogen, hydroxy, amino and the like functional group, is not critical so long as it does not adversely affect the overall activity and/or synthesis procedure. Further, it is contemplated that manganese(III) and iron (II) complexes will be equivalent to the subject manganese(II) and iron (III) complexes.

The chemical reactions described above are generally disclosed in terms of their broadest application to the preparation of the compounds of this invention. Occasionally, the reactions may not be applicable as described to each compound included within the disclosed scope. The compounds for which this occurs will be readily recognized by those skilled in the art. In all such cases, either the reactions can be successfully performed by conventional modifications known to those skilled in the art, e.g., by appropriate protection of interfering groups, by changing to

alternative conventional reagents, by routine modification of reaction conditions, and the like, or other reactions disclosed herein or otherwise conventional, will be applicable to the preparation of the corresponding compounds of this invention. In all preparative methods, all starting materials are known or readily preparable from known starting materials.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

EXAMPLES

All reagents were used as received without purification unless otherwise indicated. All NMR spectra were obtained on a Varian VXR-300 or VXR-400 nuclear magnetic resonance spectrometer. Qualitative and quantitative mass spectroscopy was run on a Finigan MAT90, a Finigan 4500 and a VG40-250T using m-nitrobenzyl alcohol (NBA), m-nitrobenzyl alcohol/LiCl (NBA - Li). Melting points (mp) are uncorrected.

The following abbreviations relating to amino acids and their protective groups are in accordance with the recommendation by IUPAC-IUB Commission on Biochemical Nomenclature (*Biochemistry* 1972, *11*, 1726) and common usage.

Ala	L-Alanine
DAla	D-Alanine
Gly	Glycine
Ser	L-Serine
DSer	D-Serine
Bzl	Benzyl
Boc	*tert*-Butoxycarbonyl
Et	Ethyl
TFA	Trifluoroacetic acid
DMF	Dimethylformamide
HOBT•H_2O	1-Hydroxy-*(1H)*-benzotriazole monohydrate
EDC•HCl	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
TEA	Triethylamine
DMSO	Dimethylsulfoxide
THF	Tetrahydrofuran
DPPA	Diphenylphosphoryl azide

*The abbreviation Cyc represents 1,2-cyclohexanediamine (stereochemistry, i.e. R,R or S,S, is indicated as such). This allows three letter code peptide nomenclature to be used in pseudopeptides containing the 1,2-cyclohexane diamine "residue".

Example 1

A. Synthesis of N-(p-toluenesulfonyl)-(R,R)-1,2-diaminocyclohexane

To a stirred solution of (R,R)-1,2-diaminocyclohexane (300 g, 2.63 mole) in CH_2Cl_2 (5.00 l) at -10°C was added a solution of p-toluenesulfonylchloride (209 g, 1.10 mole) in CH_2Cl_2 (5.00 l) dropwise over a 7 h period, maintaining the temp at -5 to -10°C. The mixture was allowed to warm to room temp while stirring overnight. The mixture was concentrated *in vacuo* to a volume of 3 l and the white

solid was removed by filtration. The solution was then washed with H_2O (10 x 1 l) and was dried over $MgSO_4$. Removal of the solvent *in vacuo* gave 286 g (97.5% yield) of the product as a yellow crystalline solid: ^{1}H NMR ($CDCl_3$) δ 0.98 - 1.27 (m, 4 H), 1.54 - 1.66 (m, 2 H), 1.81 - 1.93 (m, 2 H), 2.34 (dt, J = 4.0, 10.7 Hz, 1 H), 2.42 (s, 3 H), 2.62 (dt, J = 4.2, 9.9 Hz, 1 H), 7.29 (d, J = 8.1 Hz, 2 H), 7.77 (d, J = 8.3 Hz, 2 H); MS (LRFAB - DTT - DTE) m/z 269 $[M + H]^+$.

<u>B. Synthesis of N-(p-toluenesulfonyl)-N'-(Boc)-(R,R)-1,2-diaminocyclohexane</u>

To a stirred solution of N-(p-toluenesulfonyl)-(R,R)-1,2-diaminocyclohexane prepared as in Example 1A (256 g, 0.955 mole) in THF (1.15 l) was added a 1 N solution of aqueous NaOH (1.15 l, 1.15 mole). Di-t-butyldicarbonate (229 g, 1.05 mole) was then added and the resulting mixture was stirred overnight. The layers were separated and the aqueous layer was adjusted to pH 2 with 1 N HCl and saturated with NaCl. The aqueous solution was then extracted with CH_2Cl_2 (2 x 500 mL) and the extracts and THF layer were combined and dried over $MgSO_4$. The solvent was removed *in vacuo* to give a yellow solid. The crude product was purified by crystallization from a THF-ether-hexanes mixture to give 310 g (88.1% yield) of the product as a white crystalline solid: mp: 137 - 139°C; ^{1}H NMR ($CDCl_3$) δ 1.04 - 1.28 (m, 4 H), 1.44 (s, 9 H), 1.61 - 1.69 (m, 2 H), 1.94 - 2.01 (m, 2 H), 2.43 (s, 3 H), 2.86 (brs, 1 H), 3.30 (br d, J = 9.6 Hz, 1 H), 4.37 (br d, J = 6.7 Hz, 1 H), 5.48 (br d, J = 4.6 Hz, 1 H), 7.27 (d, J = 9.7 Hz, 2 H), 7.73 (d, J = 8.1 Hz, 2 H); MS (LRFAB, NBA - Li) m/z 375 $[M + Li]^+$.

C. Synthesis of Boc-(R,R)-Cyc(Ts)-gly-OMe

To a stirred solution of N-(p-toluenesulfonyl)-N'-(Boc)-(R,R)-1,2-diaminocyclohexane prepared as in Example 1B (310 g, 0.841 mole) in anhydrous DMF (3.11 l) at 0° C was added NaH (37.4 g - 60% in oil, 0.934 mole) in portions and the resulting mixture was stirred for 30 min. Methyl bromoacetate (142 g, 0.925 mole) was then added dropwise over 45 min and the mixture was allowed to warm to room temp while stirring overnight. After stirring for 17 h, the solvent was removed *in vacuo* and the residue was dissolved in ethyl acetate(3 l) and H_2O (1 l). The ethyl acetate solution was washed with saturated $NaHCO_3$ (1 l), saturated NaCl (500 mL) and was dried over $MgSO_4$. The solvent was removed *in vacuo* and the resulting oil was dissolved in ether. Crystallization by the addition of hexanes gave 364 g (98% yield) of the product (TLC (98:2 $CHCl_3$-MeOH/silica gel/UV detn) showed that the product contained about 5% starting material) as colorless needles: mp of pure sample 151 - 2°C ; ^1H NMR ($CDCl_3$) δ 1.11 - 1.22 (m, 4 H), 1.45 (s, 9 H), 1.64 - 1.70 (m, 3 H), 2.16 - 2.19 (m, 1 H), 2.43 (s, 3 H), 3.34 - 3.40 (m, 2 H), 3.68 (s, 3 H), 4.06 (ABq, J = 18.5 Hz, $\Delta\nu$ = 155 Hz, 2H), 4.77 (br s 1 H), 7.30 (d, J = 8.3 Hz, 2 H), 7.82 (d, J = 8.3 Hz, 2 H); MS (LRFAB, DTT - DTE) m/z 441 $[M + H]^+$.

D. Synthesis of Boc-(R,R)-Cyc(Ts)-Gly-OH

To a stirred solution of impure Boc-(R,R)-Cyc(Ts)-Gly-OMe prepared as in Example 1C (217 g, 0.492 mole) in MeOH (1.05 l) was slowly added a 2.5N solution of aqueous NaOH (295 mL, 0.737 mole) and the resulting solution was stirred for 2 h. The solvent was removed *in vacuo* and the residue was dissolved in H_2O (1.5 l). The solution was filtered to remove a small amount of solid and was washed with ether (7 x 1 l) to remove the impurity

(compound 1B) which upon drying of the combined washes over $MgSO_4$ and removal of the solvent *in vacuo* resulted in recovery of 8.37 g. The pH of the aqueous solution was then adjusted to 2 with 1 N HCl and the product was extracted with ethyl acetate (3 x 1 l). The extracts were combined, washed with saturated NaCl (500 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the residual ethyl acetate removed by coevaporation with ether (500 mL) and then CH_2Cl_2 (500 mL) to give 205 g (97.6% yield) of the product as a white foam: ^1H NMR ($CDCl_3$) δ 1.15 - 1.22 (m, 4 H), 1.48 (s, 9 H), 1.55 - 1.68 (m, 3 H), 2.12 - 2.15 (m, 1 H), 2.43 (s, 3 H), 3.41 - 3.49 (m, 2 H), 3.97 (ABq, J = 17.9 Hz, $\Delta\upsilon$ = 69.6 Hz, 2 H), 4.79 (br s, 1 H), 7.31 (d, J = 8.1 Hz, 2 H), 7.77 (d, J = 8.3 Hz, 2 H), 8.81 (br s, 1 H); MS (LRFAB, NBA - Li) m/z 433 $[M + Li]^+$.

<u>E. Synthesis of Boc-(R,R)-Cyc(Ts)-Gly-Gly-OEt</u>

To Boc-(R,R)-Cyc(Ts)-Gly-OH (18.1 g, 43.1 mmol) in DMF (480 mL) was added $HOBt\bullet H_2O$ (7.92 g, 51.7 mmol) and EDC•HCl (9.91 g, 51.7 mmol) and the resulting mixture was allowed to stir for 20 min at RT. To this solution was added GlyOEt•HCl (6.0 g, 43.1 mmol) and TEA (7.2 mL, 51.7 mmol) and the resulting mixture was allowed to stir for 16 h thereafter. The DMF was evaporated and the residue was partitioned between water (250 mL) and EtOAc (400 mL). The EtOAc layer was separated and washed with 1N $KHSO_4$ (250 mL), water (250 mL), sat. $NaHCO_3$ (250 mL) and brine (250 mL) and dried (Na_2SO_4). Filtration and concentration afforded 21.9 g (99% yield) of pure product as a white foam: ^1H NMR (DMSO-d_6) δ 1.00 - 1.10 (m, 1 H), 1.19 (t, J = 7.6 Hz, 3 H), 1.38 (s, 9 H), 1.50 - 1.56 (m, 3 H), 1.75 - 1.84 (m, 1 H), 2.38 (s, 3 H), 3.30 -3.40 (bs, 2 H), 3.75 -4.01 (complex m, 4H), 4.08 (q, J = 7.6 Hz, 2 H), 6.05 (bs, 1 H), 7.32 (d, J = 8.0 Hz, 2 H), 7.77

(d, J = 8.0 Hz, 2 H), 8.32 (t , J = 7.2 Hz, 1 H); MS(HRFAB) m/z 518.2551 (M + Li)$^+$; 518.2512 calculated for $C_{24}H_{37}N_3O_7SLi$.

F. Synthesis of Cyc(Ts)-Gly-Gly-OEt TFA salt

To a solution of Boc-Cyc(Ts)-Gly-Gly-OEt (21.2 g, 41.4 mmol) in CH_2Cl_2 (180 mL) was added TFA (44 mL) and the resulting mixture was stirred at RT for 30 min. The solution was concentrated and the residue was dissolved in ether (50 mL) and precipitated with hexanes (500 mL). The solvents were decanted and the residue was washed with 10:1 hexanes/ether (500 mL). The final residue was dried thoroughly at high vacuum to afford 20.7 g (95% yield) of the product as a tan foam: ^{1}H NMR (DMSO-d_6) δ 0.85 - 0.96 (m, 1 H), 1.03 - 1.31 (complex m, 7 H), 1.09 (t, J = 7.6 Hz, 3 H), 2.00 (m, 1 H), 2.39 (s, 3 H), 3.02 (bs, 1 H), 3.62 (m, 1 H), 3.82 - 4.05 (m, 4 H), 4.10 (q, J = 7.6, 2 H), 7.41 (d, J = 8.0 Hz, 2 H), 7.67 (d, J = 8.0 Hz, 2 H), 8.25 (bs, 3 H), 9.09 (t, J = 5.63 Hz, 1 H). MS(HRFAB) m/z 418.1990 (M -TFA + Li)$^+$; 418.1988 calculated for $C_{19}H_{29}N_3O_5S$.

G. Synthesis of Boc-Orn(Z)-Cyc(Ts)-Gly-Gly-OEt

To Boc-Orn(Z)-OH (8.37 g, 22.8 mmol) in DMF (200 mL) was added HOBt•H_2O (4.29 g, 27.4 mmol) and EDC•HCl (5.25 g, 27.4 mmol) and the resulting solution was stirred for 20 min at RT. To this solution was added Cyc(Ts)-Gly-Gly-OEt TFA salt (12.0 g, 22.8 mmol) and TEA (3.82 mL, 27.4 mmol) and stirring was maintained for 16 h thereafter. The DMF was evaporated and the residue was partitioned between water (200 mL) and EtOAc (250 mL). The ETOAc layer was separated and washed with 1N $KHSO_4$ (150 mL), water (150 mL), sat. $NaHCO_3$ (150 mL) and brine (150 mL) and dried (MgSO4). Filtration and concentration afforded 15.1 g (87 % yield) of the product as a white

foam: ^1H NMR (DMSO-d_6) δ 1.00 - 1.94 (complex m, 12 H), 1.15 (t, J = 7.4 Hz, 3 H), 2.38 (s, 3 H), 2.98 (bs, 2 H), 3.30 - 3.46 (m, 2 H), 3.70 - 3.82 (m, 4 H), 3.90 4.02 (m, 1 H), 4.05 (t, J = 7.4 Hz, 2 H), 5.00 (s, 2 H), 6.43 (m, 1 H), 7.17 (m, 1 H), 7.20 - 7.37 (m, 8 H), 7.78 (m, 2 H), 8.30 (bs, 1 H); MS(LRFAB, NBA + HCl) m/z 760 $(M + H)^+$.

H. Synthesis of Orn(Z)-Cyc(Ts)-Gly-Gly-OEt TFA salt

To a solution of Boc-Orn(Z)-Cyc(Ts)-Gly-Gly-OEt (14.5 g, 19.1 mmol) in CH_2Cl_2 (120 mL) was added TFA (30 mL) and the resulting solution was stirred at RT for 30 min. The solution was evaporated and the residue was triturated with ether (100 mL). The ether was decanted and the residue was dried thoroughly at high vacuum to afford 15.5 g (>100 % yield, contains TFA) of the product as an orange foam: ^1H NMR (DMSO-d_6) δ 0.97 - 1.93 (comples m, 12 H), 1.16 (t, J = 7.4 Hz, 3 H), 2.38 (s, 3 H), 2.98 (bs, 2 H), 3.31 - 3.50 (m, 2 H), 3.71 - 3.91 (m, 4 H), 3.97 - 4.04 (m, 1 H), 4.08 (q, J = 7.4 Hz, 2 H), 5.00 (s, 2H), 7.23 - 7.39 (m, 8 H), 7.77 - 7.81 (m, 2H), 8.18 (bs, 3 H), 8.41 (bs, 1 H); MS(LRFAB, NBA + HCl) m/z 660 $(M - TFA)^+$.

I. Synthesis of Boc-Gly-Orn(Z)-Cyc(Ts)-Gly-Gly-OEt

To a solution of Boc-Gly-OH (3.36 g, 19.2 mmol) in DMF (220 mL) was added HOBt•H_2O (3.52 g, 23.0 mmol) and EDC•HCl (4.41 g, 23.0 mmol) and the resulting solution was stirred for 20 min at RT. To this solution was added Orn(Z)-Cyc(Ts)-Gly-Gly-OEt TFA salt (14.8 g, 19.2 mmol) and TEA (3.20 mL, 23.0 mmol) and stirring was maintained for 12 h thereafter. The DMF was evaporated and the residue was partitioned between water (200 mL) and EtOAc (350 mL). The layers were separated and the EtOAc layer was washed with 1N $KHSO_4$ (150 mL), water (150 mL), sat. $NaHCO_3$ (150 mL) and brine (150 mL) and dried ($MgSO_4$).

Filtration and concentration afforded 13.7 g (87% yield) of the product as a white foam: ^{1}H NMR (DMSO-d_6) δ 0.96 - 1.10 (m, 2 H), 1.17 (t, J = 7.4 Hz, 3 H), 1.38 (s, 9H), 1.35 - 2.00 (complex m, 10 H), 2.97 (m, 2 H), 3.60 (bs, 2 H), 3.67 - 3.84 (m, 4 H), 3.93 - 4.03 (m, 3 H), 4.06 (q, J = 7.4 Hz, 2 H), 6.92 (bs, 1H), 7.19 (m, 1 H), 7.24 - 7.37 (m, 7 H), 7.60 (d, J = 8.3 Hz, 1 H), 7.76 (m, 2 H), 7.38 (bs, 1 H). MS(LRFAB, NBA + Li)$^+$ m/z 823 (M+Li)$^+$.

J. Synthesis of Boc-Gly-Orn(Z)-Cyc(Ts)-Gly-Gly-OH

To a solution of Boc-Gly-Orn(Z)-Cyc(Ts)-Gly-Gly-OEt (13.3 g, 16.3 mmol) in methanol (100 mL) was added 1 N NaOH (25 mL). The resulting mixture was stirred at RT and monitored by TLC. After 2 h the reaction was complete. The methanol was evaporated and water (50 mL) was added to the residue. This aqueous phase was washed with EtOAc (2 x 100 mL) and the EtOAc layers were discarded. The pH was lowered to 3.5 with 1N $KHSO_4$ and the aqueous phase was extracted with EtOAc (3 x 100 mL). The combined EtOAc layers were dried ($MgSO_4$), filtered and concentrated to afforded 11.7 g (91% yield) of the product as a white foam: ^{1}H NMR ($CDCl_3$) δ 0.98 - 1.25 (m, 2 H), 1.38 (s, 9 H), 1.40 - 1.92 (m, 10 H), 2.38 (s, 3 H), 2.97 (m, 2 H), 3.62 (bs, 2 H), 3.75 - 3.85 (m, 3 H), 3.95 - 4.05 (m, 2 H), 5.01 (s, 2 H), 6.96 (bs, 1 H), 7.28 (m, 1 H), 7.25 - 7.38 (m, 7 H), 7.61 (d, J = 8.4 Hz, 1 H), 7.78 (m, 2 H), 8.25 (bs, 1 H).

K. Synthesis of Gly-Orn(Z)-Cyc(Ts)-Gly-Gly-OH TFA salt

To a solution of Boc-Gly-Orn(Z)-Cyc(Ts)-Gly-Gly-OH (11.2 g, 14.3 mmol) in CH_2Cl_2 (100 mL) was added TFA (24 mL) and the resulting solution was stirred for 30 min at RT. The solution was concentrated and triturated with ethyl ether (500 mL). Filtration of afforded 11.3 g

(99% yield) of the product as a white powder: ^{1}H NMR (DMSO-d_6) δ 0.95 - 1.98 (complex m, 12 H), 2.39 (s, 3 H), 3.01 (m, 2 H), 3.38 (m, 1 H), 3.65 - 4.10 (complex m, 7 H), 4.18 (q, J = 7.4 Hz, 1 H), 5.02 (s, 2 H), 7.24 - 7.40 (m, 9 H), 7.77 - 7.85 (m, 2 H), 8.13 (bs, 3 H), 8.31 (bs, 1 H), 8.42 (d, J = 8.3 Hz, 1 H); MS(HRFAB) 689.2953 (M-TFA)$^+$; 689.2969 calculated for $C_{32}H_{45}N_6O_9S$.

L. Synthesis of *cyclo*-(Gly-Orn(Z)-Cyc(Ts)-Gly-Gly-)

A solution of Gly-Orn(Z)-Cyc(Ts)-Gly-Gly-OH TFA salt (5.0 g, 6.23 mmol) in dry degassed DMF (1520 mL) was treated with TEA (1.74 mL, 12.5 mmol) and cooled to -40°C. DPPA (1.64 mL, 7.60 mmol was added dropwise over 10 min and the reaction was stirred at -40°C for 3 hr thereafter. After this time the reaction was place in a -2°C bath and allowed to stand at this temperature for 16 h thereafter. Water (1520 mL) was added and the resulting solution was stirred with mixed bed ion-exchange resin (750 g) for 6 h at RT. The resin was filtered and the solution was concentrated to a volume of ~100 mL (DMF). The addition of ethyl ether (500 mL) produced a solid residue which was redissolved in methanol (100 mL) and again precipitated by the addition of ethyl ether (500 mL). Filtration afforded 3.26 g (78% yield) of product as a white powder: ^{1}H NMR ($CDCl_3$) δ 0.96 - 2. 10 (complex m, 14 H), 2.37 (bs, 3 H), 2.68 - 3.05 (m, 3 H), 3.42 - 3.90 (complex m, 8 H), 4.14 (m, 1 H), 4.20 (m, 1 H), 4.97 - 5.08 (m, 3 H), 6.42 (d, J = 8.4 Hz, 1 H), 7.19 (d, J = 8.0 Hz, 1 H), 7.20 - 7.39 (m, 7 H), 7.65 - 7.78 (m, 2 H), 9.15 (bs, 1 H), 9.22 (bs, 1 H); MS(HRFAB) m/z 671.2842 (M + H)$^+$; 671.2863 calculated for $C_{32}H_{43}N_6O_8S$.

M. Synthesis of *cyclo*-(Gly-Orn-Cyc(Ts)-Gly-Gly-)

To a solution of *cyclo*-(Gly-Orn(Z)-Cyc(Ts)-Gly-Gly-) (3.94 g, 5.90 mmol) in methanol (40 mL) was added Pd (black) (1.0 g) and ammonium formate (2.0 g). The reaction was refluxed for 2 h and allowed to cool. The mixture was filtered under Argon through a pad of celite and the filtrate was concentrated to afford 2.86 g (89% yield) of product as a white foam: ^{1}H NMR (DMSO-d_6) δ 0.94 - 2.22 (complex m, 12 H), 2.39 (s, 3 H), 2.55 - 2.95 (m, 7 H), 3.42 - 3.89 (complex m, 9 H), 4.11 (m, 1 H), 4.39 (m, 1 H), 6,43 (d, J = 8.4 Hz, 1 H), 7.27 (d, J = 9.3 Hz, 1 H), 7.25 - 7.45 (m, 2 H), 7.64 - 7.80 (m, 2 H), 9.12 - 9.29 (m, 2 H); MS (HRFAB) m/z 537.2511 $(M + H)^+$; 537.2495 calculated for $C_{24}H_{36}N_6SO_6$.

N. Synthesis of *cyclo*-(Gly-Orn(Lithocholyl)-Cyc(Ts)-Gly-Gly-)

To a solution of *cyclo*-(Gly-Orn-Cyc(Ts)-Gly-Gly-) (1.0 g, 1.9 mmol) in CHCl3 (25 mL) was added lithocholic acid NHS active ester (881 mg, 1,9 mmol) and the resulting mixture was stirred for 16 h thereafter. Addition of ethyl ether (50 mL) produced a solid. Filtration afforded 946 mg (56% yield) of the product as a tan powder: ^{1}H NMR (CD_3OD) δ 0.66 (m, 3 H), 0.93 (bs, 6 H), 0.94 - 2.37 (complex m, 48 H), 2.43 (s, 3H), 2.80 - 4.60 (bm, 14 H), 7.39 (bs, 2 H), 7.80 (bs, 2 H); MS (HRFAB) m/z 895.5432 (M + H)+; 895.5367 calculated for $C_{48}H_{75}N_6O_8S$.

O. Synthesis of 2,3-(R,R)-Cyclohexano-6-(S)-{3-(lithocholylamino)propyl}-1,4,7,10,13-penta-azacclopentadecane

To a suspension of *cyclo*-(Gly-Orn(Lithocholyl)-Cyc(Ts)-Gly-Gly-) (2.70 g, 3.00 mmol) in THF (50 mL) was added lithium aluminum hydride (51.0 mL of a 1.0 M solution). The resulting mixture was refluxed for 16 h

thereafter. The reaction mixture was cooled to --20°C and quenched (cautiously) with 5 % Na_2SO_4 (30 mL) followed by methanol (30 mL). This solution was stirred at RT for 1 h and concentrated to a dry powder. The powder was triturated with ethyl ether (3 x 200 mL) and filtered. The ether was concentrated and the oil was recrystallized from acetonitrile to afford 800 mg (40% yield) of product as a colorless oil: ^1H NMR (C_6D_6) δ 0.64 (s, 3 H), 0.67 (s, 3 H), 0.88 (d, J = 3.0 Hz, 3 H), 0.84 - 2.61 (complex m, 52 H), 2.38 - 2.95 (complex m, 14 H), 3.49 (m, 3 H); ^{13}C NMR ($CDCl_3$) δ 71.4, 63.1, 62.6, 61.8, 58.2, 56.5, 56.1, 51.5, 50.4, 50.1, 48.3, 47.9, 46.1, 45.7, 42.6, 42.1, 40.4, 40.1, 36.4, 35.8, 35.7, 35.6, 35.4, 34.5, 31.9, 31.7, 31.6, 30.8, 30.5,29.4, 28.3, 27.2, 26.4, 26.2, 24.9, 24.2, 23.4, 20.8, 18.6, 12.0; MS(LRFAB, NBA + Li) m/z 677 $(M+Li)^+$.

P. Synthesis of [Manganese (II) dichloro 2,3-(R,R)-Cyclohexano-6-(S)-{3-(lithocholylamino)-propyl}-1,4,7,10,13-penta-azacclopentadecane]

2,3-(R,R)-Cyclohexano-6-(S)-{3-(lithocholylamino)propyl}-1,4,7,10,13-penta-azacclopentadecane prepared as in example 10 (547 mg, 0.817 mmol) was added to a hot anhydrous methanol solution (50 mL) containing manganese (II) chloride (103 mg, 0.818 mmol) under a dry nitrogen atmosphere. After refluxing for 2 h the solution was reduced to dryness and the residue was dissolved in a solvent mixture of THF (35 mL) and ethyl ether (5 mL) and filtered through a pad of celite. Concentration and trituration with ethyl ether afforded after filtration 512 mg (79% yield) of the complex as a white solid: FAB mass spectrum (NBA) m/z 760 $[M-Cl]^+$; Anal. Calculated. for C41H78N6OMnCl2: C, 61.79; H, 9.87; N, 10.55; Cl, 8.90. Found: C, 62.67; H, 9.84; N, 8.04; Cl, 8.29.

Example 2

Stopped-Flow Kinetic Analysis

Stopped-flow kinetic analysis has been utilized to determine whether a compound can catalyze the dismutation of superoxide (Riley, D.P., Rivers, W.J. and Weiss, R.H., "Stopped-Flow Kinetic Analysis for Monitoring Superoxide Decay in Aqueous Systems," Anal. Biochem, 196, 344-349 [1991]). For the attainment of consistent and accurate measurements all reagents were biologically clean and metal-free. To achieve this, all buffers (Calbiochem) were biological grade, metal-free buffers and were handled with utensils which had been washed first with 0.1 N HCl, followed by purified water, followed by a rinse in a 10^4 M EDTA bath at pH 8, followed by a rinse with purified water and dried at 65°C for several hours. Dry DMSO solutions of potassium superoxide (Aldrich) were prepared under a dry, inert atmosphere of argon in a Vacuum Atmospheres dry glovebox using dried glassware. The DMSO solutions were prepared immediately before every stopped-flow experiment. A mortar and pestle were used to grind the yellow solid potassium superoxide (~100 mg). The powder was then ground with a few drops of DMSO and the slurry transferred to a flask containing an additional 25 ml of DMSO. The resultant slurry was stirred for 1/2 h and then filtered. This procedure gave reproducibly ~2 mM concentrations of superoxide in DMSO. These solutions were transferred to a glovebag under nitrogen in sealed vials prior to loading the syringe under nitrogen. It should be noted that the DMSO/superoxide solutions are extremely sensitive to water, heat, air, and extraneous metals. A fresh, pure solution has a very slight yellowish tint.

Water for buffer solutions was delivered from an in-house deionized water system to a Barnstead Nanopure Ultrapure Series 550 water system and then double

distilled, first from alkaline potassium permanganate and then from a dilute EDTA solution. For example, a solution containing 1.0 g of potassium permanganate, 2 liters of water and additional sodium hydroxide necessary to bring the pH to 9.0 were added to a 2-liter flask fitted with a solvent distillation head. This distillation will oxidize any trace of organic compounds in the water. The final distillation was carried out under nitrogen in a 2.5-liter flask containing 1500 ml of water from the first still and 1.0×10^{6} M EDTA. This step will remove remaining trace metals from the ultrapure water. To prevent EDTA mist from volatilizing over the reflux arm to the still head, the 40-cm vertical arm was packed with glass beads and wrapped with insulation. This system produces deoxygenated water that can be measured to have a conductivity of less than 2.0 nanomhos/cm^2.

The stopped-flow spectrometer system was designed and manufactured by Kinetic Instruments Inc. (Ann Arbor, MI) and was interfaced to a MAC IICX personal computer. The software for the stopped-flow analysis was provided by Kinetics Instrument Inc. and was written in QuickBasic with MacAdios drivers. Typical injector volumes (0.10 ml of buffer and 0.006 ml of DMSO) were calibrated so that a large excess of water over the DMSO solution were mixed together. The actual ratio was approximately 19/1 so that the initial concentration of superoxide in the aqueous solution was in the range 60-120 μM. Since the published extinction coefficient of superoxide in H_2O at 245 nm is ~2250 M^{-1} cm^{-1} (1), an initial absorbance value of approximately 0.3-0.5 would be expected for a 2-cm path length cell, and this was observed experimentally. Aqueous solutions to be mixed with the DMSO solution of superoxide were prepared using 80 mM concentrations of the Hepes buffer, pH 8.1 (free acid + Na form). One of the reservoir syringes was filled with 5 ml of the DMSO

solution while the other was filled with 5 ml of the aqueous buffer solution. The entire injection block, mixer, and spectrometer cell were immersed in a thermostatted circulating water bath with a temperature of 21.0 ± 0.5°C.

Prior to initiating data collection for a superoxide decay, a baseline average was obtained by injecting several shots of the buffer and DMSO solutions into the mixing chamber. These shots were averaged and stored as the baseline. The first shots to be collected during a series of runs were with aqueous solutions that did not contain catalyst. This assures that each series of trials were free of contamination capable of generating first-order superoxide decay profiles. If the decays observed for several shots of the buffer solution were second-order, solutions of manganese(II) complexes could be utilized. In general, the potential SOD catalyst was screened over a wide range of concentrations. Since the initial concentration of superoxide upon mixing the DMSO with the aqueous buffer was ~1.2×10^{-4} M, we wanted to use a manganese (II) complex concentration that was at least 20 times less than the substrate superoxide. Consequently, we generally screened compounds for SOD activity using concentrations ranging from 5×10^{-7} to 8×10^{-6} M. Data acquired from the experiment was imported into a suitable math program (e.g., Cricket Graph) so that standard kinetic data analyses could be performed.

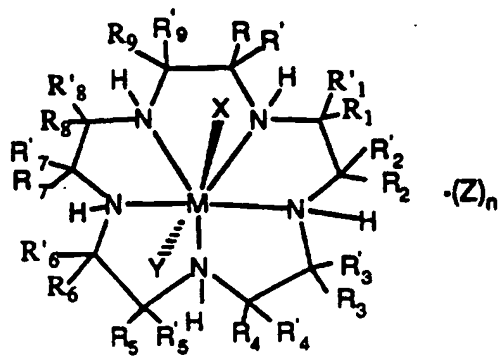
The catalytic rate constant for dismutation of superoxide by the manganese(II) complex of Example 1 was determined from the linear plot of observed rate constants (k_{obs}) versus the concentration of the manganese(II) complexes. k_{obs} values were obtained from the liner plots of ln absorbance at 245 nm versus time for the dismutation of superoxide by the manganese(II) complex. The k_{cat} ($M^{-1}sec^{-1}$) of the manganese (II) complex of Example 1 at

pH = 8.1 and 21°C was determined to be $0.77 \times 10^{+7}$ $M^{-1}sec^{-1}$.

The manganese(II) complex of the nitrogen-containing macrocyclic ligand in Example 1 is an effective catalyst for the dismutation of superoxide, as can be seen from the k_{cat} above.

WHAT IS CLAIMED IS:

1. A compound which is a complex represented by the formula:



wherein R, R', R_1, R'_1, R_2, R'_2, R_3, R'_3 R_4, R'_4, R_5, R'_5, R_6, R'_6, R_7, R'_7, R_8, R'_8, R_9 and R'_9 independently represents alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkylalkyl, cycloalkylcycloalkyl, cycloalkenylalkyl, alkylcycloalkyl, alkenylcycloalkyl, alkylcycloalkenyl, alkenylcycloalkenyl, heterocyclic, aryl and aralkyl radicals and radicals attached to the α-carbon of α-amino acids; or R_1 or R'_1 and R_2 or R'_2, R_3 or R'_3 and R_4 or R'_4, R_5 or R'_5 and R_6 or R'_6, R_7 or R'_7 and R_8 or R'_8, and R_9 or R'_9 and R or R′ together with the carbon atoms to which they are attached independently form a saturated, partially saturated or unsaturated cyclic having 3 to 20 carbon atoms; or R or R′ and R_1 or R'_1, R_2 or R'_2 and R_3 or R'_3, R_4 or R'_4 and R_5 or R'_5, R_6 or R'_6 and R_7 or R'_7, and R_8 or R'_8 and R_9 or R'_9 together with the carbon atoms to which they are attached independently form a nitrogen containing heterocycle having 2 to 20 carbon atoms provided that when the nitrogen containing heterocycle is an aromatic heterocycle which does not contain a hydrogen attached to the nitrogen, the hydrogen attach d to the nitrogen in said formula, which nitrogen

is also in the macrocycle and the R groups attached to the same carbon atoms of the macrocycle are absent; and combinations thereof;

wherein (1) one to five of the "R" groups are attached to biomolecules via a linker group, (2) one of X, Y and Z is attached to a biomolecule via a linker group, or (3) one to five of the "R" groups and one of X, Y and Z are attached to biomolecules via a linker group; and said biomolecules are independently selected from the group consisting of steroids, carbohydrates, fatty acids, amino acids, peptides, proteins, antibodies, vitamins, lipids, phospholipids, phosphates, phosphonates, nucleic acids, enzyme substrates, enzyme inhibitors and enzyme receptor substrates and said linker group is derived from a substituent attached to said "R" group or said X, Y and Z which is reactive with the biomolecule and is selected from the group consisting of $-NH_2$, $-NHR_{10}$, -SH, -OH, -COOH, $-COOR_{10}$, $-CONH_2$, -NCO, -NCS, -COOX", alkenyl, alkynyl, halide, tosylate, mesylate, tresylate, triflate and phenol, wherein R_{10} is alkyl, aryl or alkaryl and X" is a halide; wherein M is Mn or Fe; and wherein X,Y and Z are ligands independently selected from the group consisting of halide, oxo, aquo, hydroxo, alcohol, phenol, dioxygen, peroxo, hydroperoxo, alkylperoxo, arylperoxo, ammonia, alkylamino, arylamino, heterocycloalkyl amino, heterocycloaryl amino, amine oxides, hydrazine, alkyl hydrazine, aryl hydrazine, nitric oxide, cyanide, cyanate, thiocyanate, isocyanate, isothiocyanate, alkyl nitrile, aryl nitrile, alkyl isonitrile, aryl isonitrile, nitrate, nitrite, azido, alkyl sulfonic acid, aryl sulfonic acid, alkyl sulfoxide, aryl sulfoxide, alkyl aryl sulfoxide, alkyl sulfenic acid, aryl sulfenic acid, alkyl sulfinic acid, aryl sulfinic acid, alkyl thiol carboxylic acid, aryl thiol carboxylic acid, alkyl thiol thiocarboxylic acid, aryl thiol thiocarboxylic acid, alkyl carboxylic acid, aryl

carboxylic acid, urea, alkyl urea, aryl urea, alkyl aryl urea, thiourea, alkyl thiourea, aryl thiourea,alkyl aryl thiourea, sulfate, sulfite, bisulfate, bisulfite, thiosulfate, thiosulfite, hydrosulfite, alkyl phosphine, aryl phosphine, alkyl phosphine oxide, aryl phosphine oxide, alkyl aryl phosphine oxide, alkyl phosphine sulfide, aryl phosphine sulfide, alkyl aryl phosphine sulfide, alkyl phosphonic acid, aryl phosphonic acid, alkyl phosphinic acid, aryl phosphinic acid, alkyl phosphinous acid, aryl phosphinous acid, phosphate, thiophosphate, phosphite, pyrophosphite, triphosphate, hydrogen phosphate, dihydrogen phosphate, alkyl guanidino, aryl guanidino, alkyl aryl guanidino, alkyl carbamate, aryl carbamate, alkyl aryl carbamate, alkyl thiocarbamate, aryl thiocarbamate, alkyl thiocarbamate, alkyl dithiocarbamate, aryl dithiocarbamate, alkylaryl dithiocarbamate, bicarbonate, carbonate, perchlorate, chlorate, chlorite, hypochlorite, perbromate, bromate, bromite, hypobromite, tetrahalomanganate, tetrafluoroborate, hexafluoroantimonate, hypophosphite, iodate, periodate, metaborate, tetraaryl borate, tetra alkyl borate, tartrate, salicylate, succinate, citrate, ascorbate, saccharinate, amino acid, hydroxamic acid, thiotosylate, and anions of ion exchange resins, or the corresponding anions thereof, or X, Y and Z are independently attached to one or more of the "R" groups and n is 0 or 1.

2. Compound of Claim 1 wherein 1 to 2 of the "R" groups are attached to biomolecules via a linker group and none of X, Y and Z is attached to a biomolecule via a linker group.

3. Compound of Claim 1 wherein one of X, Y and Z is attached to a biomolecule via a linker group and none of the "R" groups are attached to biomolecules via a linker group.

4. Compound of Claim 1 wherein a maximum of one

"R" group attached to the carbon atoms of the macrocycle located between nitrogen atoms has a biomolecule attached via a linker group.

5. Compound of Claim 1 wherein at least one of the "R" groups, in addition to the "R" groups which are attached to biomolecules via a linker group, are independently selected from the group consisting of alkyl, cycloalkyl, cycloalkylalkyl, aralkyl, alkary, aryl, heterocyclics and radicals attached to the α-carbon of α-amino acids, and the remaining "R" groups are independently selected from hydrogen, saturated, partially saturated or unsaturated cyclics or a nitrogen containing heterocycle.

6. Compound of Claim 5 wherein at least two of the "R" groups, in addition to the "R" groups which are attached to biomolecules via a linker group, are independently selected from the group consisting of alkyl, cycloalkyl, cycloalkylalkyl, aralkyl, alkaryl, aryl, heterocyclics and radicals attached to the α-carbon of α-amino acids.

7. Compound of Claim 5 wherein at least one of the "R" groups, in addition to the "R" groups which are attached to biomolecules, via a linker group, are alkyl and the remaining "R" groups are independently selected from hydrogen or saturated, partially saturated or unsaturated cyclics.

8. Compound of Claim 1 wherein at least one of R_1 or R'_1 and R_2 or R'_2, R_3 or R'_3 and R_4 or R'_4, R_5 or R'_5 and R_6 or R'_6, R_7 or R'_7 and R_8 or R'_8, and R_9 or R'_9 and R or R' together with the carbon atoms to which they are attached represent a saturated, partially saturated or unsaturated cyclic having 3 to 20 carbon atoms and the remaining "R" groups in addition to the "R" groups which are attached to biomolecules via linker groups are independently selected from hydrogen, nitrogen containing heterocycles or alkyl groups.

9. Compound of Claim 8 wherein at least two of R_1 or R'_1 and R_2 or R'_2, R_3 or R'_3 and R_4 or R'_4, R_5 or R'_5 and R_6 or R'_6, R_7 or R'_7 and R_8 or R'_8, and R_9 or R'_9 and R or R' together with the carbon atoms to which they are attached represent a saturated, partially saturated or unsaturated cyclic having 3 to 20 carbon atoms and the remaining "R" groups in addition to the "R" groups which are attached to biomolecules via linker groups are independently selected from hydrogen, nitrogen containing heterocycles or alkyl groups.

10. Compound of Claim 8 wherein said saturated, partially saturated or unsaturated cyclic is cyclohexyl.

11. Compound of Claim 10 wherein said remaining "R" groups in addition to the "R" groups which are attached to biomolecules via linker groups are independently selected from hydrogen or alkyl groups.

12. Compound of Claim 1 wherein said R or R' and R_1 or R_1', R_2 or R_2' and R_3 or R_3', R_4 or R'_4 and R_5 or R_5', R_6 or R_6' and R_7 or R_7', and R_8 or R'_8 and R_9 or R_9' together with the carbon atoms to which they are attached are found to form a nitrogen containing heterocycle having 2 to 20 carbon atoms, and the remaining "R" groups in addition to the "R" groups which are attached to biomolecules via a linker group are independently selected from hydrogen, saturated, partially saturated or unsaturated cyclics or alkyl groups.

13. Compound of Claim 1 wherein X, Y and Z are independently selected from the group consisting of halide, organic acid, nitrate and bicarbonate anions.

14. Compound of Claim 1 wherein M is Fe.

15. Compound of Claim 1 wherein M is Mn.

16. Pharmaceutical composition in unit dosage form useful for dismutating superoxide comprising (a) a therapeutically or prophylactically effective amount of a complex of Claim 1 and (b) a nontoxic, pharmaceutically acc ptable carrier, adjuvant or vehicle.

17. Method of preventing or treating a disease or disorder which is mediated, at least in part, by superoxide comprising administering to a subject in need of such prevention or treatment, a therapeutically, prophylactically, pathologically, or resuscitative effective amount of a complex of Claim 1.

18. Method of Claim 17 wherein said disease or disorder is selected from the group consisting of ischemic reperfusion injury, surgically-induced ischemia, inflammatory bowel disease, rheumatoid arthritis, atherosclerosis, thrombosis, platelet aggregation, oxidant-induced tissue injuries and damage, osteoarthritis, psoriasis, organ transplant rejections, radiation-induced injury, stroke,acute pancreatitis, insulin-dependent diabetes mellitus, adult and infantile respiratory distress, metastasis and carcinogenesis.

19. Method of Claim 18 wherein said disease or disorder is selected from the group consisting of ischemic reperfusion injury, stroke, atherosclerosis and inflammatory bowel disease.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/02566

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D259/00 A61K31/555

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 524 161 A (MONSANTO) 20 January 1993 see whole document ---	1-19
Y	WO 95 10185 A (DUKE UNIVERSITY) 20 April 1995 see whole document ---	1-19
Y	WO 93 11800 A (DOW CHEMICAL CO.) 24 June 1993 see claims ---	1-19
P,Y	WO 96 39396 A (MONSANTO) 12 December 1996 see claims; examples ---	1-19
P,Y	WO 96 39409 A (NITROMED INC.) 12 December 1996 see claims; examples ---	1-19
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
4 July 1997	28.07.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Helps, I

Form PCT/ISA/210 (second sheet) (July 1992)

International Application No
PCT/US 97/02566

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	WO 96 40658 A (MONSANTO) 19 December 1996 see claims; examples -----	1-19

2

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

International application No.
PCT/US 97/02566

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 17-19
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 17-19 are drawn to a method of treatment of the human or animal body by therapy (Rule 39-1(IV)PCT), the search has been carried out based on the alleged effects of the compounds/compositions

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/02566

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 524161 A	20-01-93	AU 661023 B	13-07-95
		AU 2338392 A	23-02-93
		CA 2072897 A	20-01-93
		CA 2072934 A	20-01-93
		EP 0598753 A	01-06-94
		JP 6509566 T	27-10-94
		NZ 272364 A	27-02-96
		WO 9302090 A	04-02-93
		US 5637578 A	10-06-97
		US 5610293 A	11-03-97
		ZA 9205139 A	26-04-93
WO 9510185 A	20-04-95	AU 7976394 A	04-05-95
		CA 2174236 A	20-04-95
		EP 0723398 A	31-07-96
WO 9311800 A	24-06-93	AU 3415493 A	19-07-93
		CA 2103554 A	11-06-93
		EP 0579802 A	26-01-94
		HU 67602 A	28-04-95
		JP 7502277 T	09-03-95
		US 5480990 A	02-01-96
		ZA 9209575 A	10-06-94
WO 9639396 A	12-12-96	AU 6023796 A	24-12-96
WO 9639409 A	12-12-96	AU 6031096 A	24-12-96
WO 9640658 A	19-12-96	AU 5928396 A	30-12-96